**LEGUME INNOVATION LAB FOR COLLABORATIVE RESEARCH ON GRAIN LEGUMES**

**FY 2016 WORKPLAN**

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

**Lead U.S. Principal Investigator (PI) and affiliated Lead U.S. University:**
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**I. Project Problem Statement and Justification:**
The primary 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 with desirable grain characteristics; and 4) provide training and capacity building in modern cowpea breeding. In addressing these primary constraints, the objectives are well-aligned with Feed The Future research strategic priorities of 1) crop resistance to heat, drought, salinity and flood; 2) West African Sudano-Sahelian systems with emphasis on insect-resistant cowpea; and 3) grain legume productivity. Our plan includes the FTF focus countries Ghana and Senegal, and also Burkina Faso, which offers regional importance from an agro-ecological perspective for cowpea yield gain in the Sudano-Sahel region. Strategically, these countries represent the primary agro-ecologies underpinning cowpea production in this region.

We will employ genomics and modern breeding methods to improve cowpea for yield limiting constraints. By leveraging genomic resources developed under complementary cowpea genomics and modern breeding work funded by the CGIAR Generation Challenge Program and USAID Innovation Lab for Climate Resilient Cowpea, we will apply comprehensive modern breeding tools and methods for genetic improvement of cowpea emphasizing insect tolerance and resistance. Insect pests are seen as a major constraint to cowpea productivity in West Africa. The project team has determined that significant gain can be made by targeting the major insect threats that occur at early (aphids), mid-flowering and pod-set (flower thrips), and later pod-filling (pod-sucking bugs) stages of the cowpea season. Although discovery work through phenotyping, genetic mapping and QTL identification needs to be done in most cases for these insect pests, some progress on resistance and tolerance donors and initial QTL discovery provide good starting points in the project. High-throughput SNP genotyping platforms, high density consensus cowpea genetic maps, plus numerous discovered QTL for important biotic stress resistance and abiotic drought tolerance traits are now available through our work. We are completely familiar with these technological

advancements and have experience in their application to modern cowpea breeding. We are also working closely with the CGIAR-GCP Integrated Breeding Platform – Breeding Management System development using our cowpea data as a test user case, and bring these technologies into the project work. The project breeding programs have a range of early generation populations carrying various target traits, providing valuable resources for breeding advancement.

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. Cowpea varieties with increased productivity (yield per unit area) without the need for purchased inputs especially benefit poor farmers, many being women who lack access to the most productive lands. By targeting insect tolerance and combining with drought tolerance, we have the opportunity to increase cowpea productivity. Productivity is key to increasing rural incomes and new resources can then be invested in other activities that help boost total family income. Productivity increases also help reduce prices to urban consumers. Sustainable increases in cowpea productivity in Africa and the US can be achieved through development of varieties with resistance to insects, nematodes and pathogens, Striga, drought tolerance, and the ability to thrive under of low soil fertility.

To increase marketing options, new cowpea varieties must have features desired by consumers; grain appearance, cooking and processing characteristics are especially important. Large white grains with rough seed-coat are good for direct dry-milling, and can be marketed over a wide area, buffering supply and prices in the region. Regionally adapted cowpea varieties with large white grain and resistance to pests would increase the marketing opportunities of cowpea farmers and traders in both West Africa and the US. Considerable demand exists for large rough brown grain types, especially in the large urban centers and command a premium price. However standard varieties like 'Ife Brown' are susceptible to pests and diseases and require improvement.

**II. Planned Project Activities for the Workplan Period** (October 1, 2015 – September 30, 2016)

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

**Collaborators:** Dr. Bao Lam Huynh, UC Riverside, USA
Dr. Clementine Dabire, INERA, Burkina Faso
Dr. Isgouhi Kaloshian, UC Riverside, USA
Dr. Barry Pittendrigh, U Illinois, USA
Dr. Manu Tamo, IITA, Benin
Dr. Christian Fatokun, IITA, Nigeria
Dr. Ousmane Boukar, IITA, Nigeria
Dr. Ibrahima Sarr, ISRA, Senegal

**Approaches and Methods:**
**Overall approach to sub-objectives:** We have developed the necessary tools to exploit molecular breeding for cowpea. We have also worked with the CGIAR-GCP to develop a publicly available integrated breeding platform, essentially a pipeline for conducting marker-based selection from initial crossing to new variety release. Requisite tools developed include genic SNP markers, high density SNP-based genetic maps including consensus maps using African cowpea germplasm for sub-Saharan Africa

relevant breeding use, a high-throughput SNP genotyping platform for cowpea, with conversion to a format provided through an outsource genotyping service, QTL for many major biotic and abiotic stress resistance and tolerance traits (drought, heat, fungal, bacterial and viral diseases, some insects, nematodes, Striga), and accompanying software programs. These tools, documented in the Technical Application, enable selection of multiple traits simultaneously across the genome (rather than single marker-trait selection). We will apply these technologies to existing and new breeding populations, for both QTL discovery and breeding.

Breeding targets for Africa will be to develop and release varieties that have preferred large white grain type for both domestic and export markets, and rough brown types primarily for domestic markets. The primary traits for grain yield enhancement include QTL for tolerance or resistance to three target insect pests. We have already identified a series of QTL controlling biotic and abiotic stresses. We have selected parent combinations and initiated breeding populations from their crosses which will enable selection for progeny carrying combinations of the insect tolerance with the other traits (specifically drought tolerance, nematode, Striga and Macrophomina resistance and also some virus resistance). In California, QTL for resistance to Fusarium wilt (*Fot3-1, Fot4-1, Fot4-2*), aphids (*QAc-vu1.1*, *QAc-vu7.1*) and root-knot nematodes (*Rk, Rk2, Rkn*) will be bred in backgrounds with Lygus bug tolerance, targeting the primary biotic stress constraints to yield.

Three sub-objectives focus on aphid resistance (Obj. 1.1), flower thrips resistance (Obj. 1.2), and pod-sucking bug resistance (Obj. 1.3). Each of these foci has the same goal, to discover and validate QTL underlying the target insect tolerance/resistance traits, then to apply the QTL knowledge to breeding population development and advancement, leading to enhanced yield performance cowpea varieties.

**Genotyping approach:** We will apply a combination of the KASP SNP platform that we developed with the GCP IBP and LGC KBioscience and the new 49,000 Illumina iSelect SNP platform for genotyping both in the QTL discovery phase and for breeding. The Illumina iSelect SNP platform with its high density marker resource is being applied in FY15 and will be applied during FY16 to parents and derived mapping populations focusing on the QTL discovery phase. This platform is fixed for cost per sample run using all SNPs and requires local DNA extraction, although discussion is underway to convert the mapped SNPs to a KASP platform format. The KASP platform has 1022 mapped SNPs providing excellent coverage across the cowpea genome. For cost efficiency, on a cost per data-point basis we can choose the number of SNPs to be tested on the number of genotypes needed for each QTL discovery population or breeding decision. Using genotype profiles of the parents with all SNPs, the subset of polymorphic SNPs for a desired pair of parents are selected and used to genotype the progenies (individuals or bulked families), thereby building in cost-efficiency. Our ability to choose the cM distance of markers across the genome (for background selection) and specifically QTL flanking markers for the population-specific SNP marker subsets (using our in-house 'SNP selector' program available at Breedit.org) from the polymorphic subsets, efficient genetic gain by pyramiding the target traits can be made. This approach can be used for backcross populations to select the appropriate individuals (BC1F1 or BC2F1, etc.) carrying positive alleles for making the next backcross. We will employ this genotyping approach in the workplan period. The NARS breeders will grow plants in the host country, then either take leaf punches at the young plant stage, place in 96-well plates, dehydrate with silica gel and then express ship to LGC KBioscience in the UK or USA, or for the Illumina iSelect, send dried whole leaf samples to UCR in silica gel-packed bags where DNA will be extracted and send to USC for genotyping. The

genotyping data will be analyzed and jointly interpreted for a breeding decision (which plants to use for crossing or to advance) or for QTL discovery. Iterative rounds of genotyping and periodic phenotyping to validate will be used to foreground select the desired complement of positive QTL.

**Phenotyping and data handling approach**: Phenotyping will be conducted under field, greenhouse and lab conditions (insect screens) at NARS locations using standard test protocols. Phenotypic data analyses will be by standard ANOVA. When drought tolerance is being selected, performance testing under water-limited conditions will be done at NARS field sites. Sites and protocols will be determined by the target insect pest (see below). We will use the CGIAR IBP Breeding Management System tools for data recording, processing and archiving. The variables will include geographical coordinates and dates of each trial, soil and weather data, persons conducting experiments, trait dictionary language and other parameters set up in the IBP FieldBook (tool for software tablets). This data capture format allows for export into the ICI mapping, Optimas and Backcross programs for QTL analyses and molecular score selection indices. These tools are now familiar to the project team members in Burkina Faso, Ghana, Senegal and UC-Riverside from their use in the TL1 project and through hands-on experience during FY14 and FY15 (short-term training workshops and adoption).

**1.1 Aphid resistance:** We will test 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 spring 2015. A uniform test design and coordination planning for the aphid resistance assessment was developed by the project team in FY13 – FY14. Additional germplasm lines are included in the screening sites to search for more sources of resistance. 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, although some sites had insufficient aphid infestation. This multi-site phenotype screening for resistance response is being repeated in FY15 starting in July. Following additional seed increases, the tests will be conducted in main season FY16 to provide a minimum of 2-3 years of data. The resistance donors and susceptible controls were SNP genotyped in FY14, coordinated by UCR. 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 will be collected and stored for DNA and RNA extraction (using RNAlater kits), with a view to developing sequence and expression-based profiles to distinguish the isolates. We will also be advised by Dr. Kaloshian at UCR who has been working on the complete aphid genome sequence.

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 have completed a QTL discovery effort for aphid resistance in IT97K-556-6, identifying one major and one minor QTL, to which other resistance sources will be compared. In Burkina, we have an F2 population between a susceptible elite line and resistance donor KvX295-2-124-99. For QTL mapping, this population is being advanced to F3 families in FY15 and will be

phenotyped and genotyped in FY16. From the wild donor IITA line TVNu1158 a RIL population has been developed for mapping QTL and seed increase is underway in FY15. We plan on phenotyping and genotyping this population in FY16. This work is being planned in collaboration with Dr. Fatokun at IITA, Nigeria. The QTL will be included in foreground selection in the breeding populations, with a plan to target effective resistance sources within a given NARS region (i.e., match effective resistance with preferred and adapted cowpea types for the relevant production area).

**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 and Ghana both RIL populations will be field-phenotyped for tolerance to flower Thrips using the Jackai and Singh (1988) tolerance scale, at sites in Bambey, Nioro, Tamale and Manga during the FY15 and FY16 workplan periods. This will provide multiple years and locations of phenotyping data. Additional germplasm lines will be included in the screening sites to search for more sources of resistance in both FY15 and FY16. Screens will be designed as a 4-replication RCBD and include the parents, and run by entomologists Ibrahima Sarr (Senegal) and Francis Kusi (Ghana). In Senegal the different tolerance sources in Sanzi, 58-77 and Tvx3236 were intercrossed in all combinations by Dr. Cisse in FY14; these populations are being advanced to F3 in FY15 and to F6 in FY16 and phenotyped. These sources of resistance have poor seed quality, so the F1s of their intercrosses were also crossed with the new large-seeded varieties in FY15. These will be advanced to F4 in FY16. In Ghana, three Sanzi-derived F7 populations segregating for seed color (including white) and flower thrips resistance are available 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. The SARI team will phenotype the three F7 populations for thrips tolerance in the FY15 workplan main season using the previously described experimental protocols. This will provide a second year of phenotyping data. The F3 families will be SNP genotyped using bulked leaf disks from 20 plants per family. Depending on progress, we will focus on the IT97K-499-35-derived population for improvement. In Ghana, to match the genotyping and phenotyping data of the Thrips /aphid/Striga populations, leaf samples of 272 single seed families have been sampled for SNP genotyping and the plants currently been giving the needed care and protection to produce enough seeds for phenotyping. The FY16 workplan will therefore be concentrated on the results of both the SNP genotyping and field phenotyping. The selected individuals from the IT97K-499-35 (Songotra) derived population that will combine Striga and Thrips will be further evaluated. The seeds of the selected families will be increased to enable availability of enough seeds for further evaluation.

**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 (maternal, F3) on F3 plants are being genotyped and phenotyped in FY15 to identify the

underlying QTL, using standard screens of young pods in petri dishes to score bug viability and fecundity. The phenotyping will be repeated in FY16 to provide validated QTL mapping data. Additional potential tolerance donor lines are included in the initial phenotyping screens in FY15, 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 Kvx771-10 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 FY15 and FY16 for pod bug resistance in Burkina Faso, in collaboration with Dr. Dabire. The F2 were advanced to F3 in FY15 to provide screening resources for FY15 and FY16. 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 are being genotyped in FY15. 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 will collaborate 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 and RNA collection, in which insects are placed in plastic bags with silica gel packs or in RNAlater Qiagen) kits; the former dries the insect samples and preserves the DNA, the latter preserves RNA integrity. Tests on aphid DNA with primers for the COX1 gene demonstrated excellent DNA integrity.

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

**Collaborators:** Dr. Bao Lam Huynh, UC Riverside, USA
Dr. G. McClaren, CGIAR GCP IBP
Dr. Ousmane Boukar, IITA, Nigeria
Dr. TJ Higgins, CSIRO, Canberra, Australia
Dr. Prince Addae, AATF, Nigeria
Dr. Samba Thiaw, ISRA, Senegal
Dr. Mywish Maredia, Michigan State U., USA

**Approaches and Methods:**
**2.1.** We will continue to use our genotyping capability to advance the BT gene introgression for Maruca 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, Nafi, and IT98K-205-8). We are genotyping Burkina Faso BC5 and Ghana BC2 progenies in FY15 and our plan for FY16 is to continue with additional rounds of SNP genotyping on the next generation of breeding lines. In FY15 the new 49,000-SNP Illumina iSelect genotyping panel developed under the USAID Innovation Lab for Climate Resilient Cowpea will be applied to the most advanced BC lines for selection. 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 and Dr. Prince Addae, Project Manager of AATF, Abuja, Nigeria, received extensive hands-on training at UCR in March 2014 and 2015, and they will be further trained in using their own datasets under this objective. The genotyping will mostly follow the same protocol as outlined under the Objective 1 work. We will use leaf samples from young screenhouse grown plants in the phenotyping and crossing blocks for DNA extraction in Burkina Faso and Ghana. Following shipping, the DNA samples will be SNP assayed by LGC Genomics for KASP or USC for iSelect and the genotype data sent to UCR for quality checking. The genotype data will be analyzed for molecular scores using Backcross Selector software. In Ghana, DNA extraction has been completed to conduct genotyping in FY15 –FY16.

**2.2.** We plan to capitalize 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. Specifically, in Senegal five large white grain type cowpeas (new variety names Lisard, Thieye, Leona, Kelle and Sam, with at least 25 g /100 grains) developed by Dr. Cisse are being processed for release approval by the national variety release committee during 2015. 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). Dr. Cisse will continue with Foundation Seed production in the FY15 and FY16 seasons using sites at Bambey. The Foundation Seed will be used by Certified Seed producers in the main season 2016, with training inputs from Dr. Cisse. BC4F3 lines of Melakh with Striga resistance will be available for evaluation during FY 16 main season.

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 are being re-evaluated in 2014. Multi-location tests are being used at Saria, Pobe, and Kamboinse in Burkina Faso. The best performing of the nine lines are being re-evaluated in FY15, emphasizing yield and grain quality, plus any disease susceptibility in trials using 4-row plots, 5 m long and 4 reps arranged in a RCBD. The release petitions to the national variety release committee will be made in 2015 or 2016 depending on the committee meeting schedule. Breeder Seed of the best lines chosen for release submission based on main season 2014 and off-season 2015 performance data will be produced at Saria during the main season 2015 (June – October). The Breeder Seed will be used to initiate Foundation Seed production in the FY16 off-season and start Certified Seed production in the FY16 main season.

In California, we will field test advanced breeding lines for release potential, based on performance data collected in 2015. These represent CRSP developed lines and they require at least one year more of field performance testing. The lines carry a combination of lygus bug tolerance, and root-knot nematode and Fusarium wilt resistance. For the best advanced blackeyes from 2015, we will conduct on-farm yield strip trials in a Tulare Co. farmer's field to assess commercial yield performance. The lines also will be tested at the Kearney field station (Fresno Co.). The test design will be four-row 4-fold replicated RCBD trials with the center two rows machine harvested. Yield weights, 100-seed weights and lygus damage to seed will be assayed. All yield and performance data will be analyzed by standard ANOVA.

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. 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.

**Collaborators:** Dr. Bao Lam Huynh, UC Riverside
Dr. G. McClaren, CGIAR GCP IBP
Dr. Ousmane Boukar, IITA, Nigeria

**Approaches and Methods:**
Short-term Training: Molecular breeding for young trainee breeders and NARS scientists will be conducted. Continuous short-term training will occur 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 2014 and again 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 training modules development. We will use the same format for the workshop in FY16, to be held in Livingstone, Zambia in February preceding the World Cowpea Conference. 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 will also be used by the NARS breeders to train the technical staff in the NARS programs after NARS breeders have been trained further on the standardized electronic fieldbook, leaf assay, and field phenotyping protocols.

Degree Training: We plan to conduct degree training for two graduate students in the workplan period:

1. Arsenio Ndeve, Mozambique, male student in PhD Plant Pathology program at UC Riverside, working in pathology, genetics and breeding of SE African cowpea germplasm.
2. Sassoum Lo, Senegal, female student in MS Plant Genetics program at UC Riverside, working in genomics and breeding of cowpea seed traits.

## III. Contribution of Project to USAID Feed the Future Performance Indicators:

Please see the attached completed “Performance Indicators – Targets” form for FY 2013, 2014, 2015, 2016 and 2017.

## IV. Outputs:

Under Objective 1.1 -- Aphid resistance
A differential cowpea panel of aphid resistance sources and control lines seed-

multiplied for multi-location field screening (Project team).
Molecular characterization of aphid populations collected from multiple locations.
Discovery of the extent of aphid biotype differences across four partner locations.

Under Objective 1.2 -- Thrips resistance
Two RIL populations will be phenotyped for QTL refinement in Senegal and Ghana.
F3s will be generated from thrips resistance sources intercrosses (Senegal).
Data from phenotyping 3 F7 populations with Sanzi donor parent (Ghana).
Genotyping data from F3 populations with Sanzi donor (UCR).

Under Objective 1.3 – Pod bug resistance
Data generated from genotyping parents, F2 and F3 populations derived from resistance donor IT86D-716 (UCR).
Two F3 populations developed from existing F2 for pod bug resistance (BF).
Data from phenotyping 2 F3 populations with IT86D-716 donor parent (BF).
Initial QTL from IT86D-716 discovered by ICI Mapping (UCR and BF).

Under Objective 2.1 – SNP markers for Bt introgression
Genotype data produced from Burkina Faso and Ghana Bt-transgene segregating populations (UCR).
Selection of advanced BC lines with Bt-transgene (BF and Ghana).

Under Objective 2.2 – Variety releases
Foundation and Certified Seed of 5 large white-seeded CRSP varieties in Senegal.
Breeder Seed and Foundation Seed produced of the best candidates of 9 pre-release CRSP lines evaluated in on-farm trials in FY14 to FY16 (BF).
Farmer-field strip trial performance data on California blackeye pre-release lines.

Under Objective 3 – capacity Building
Degree training of two African graduate students (UCR and Senegal).
Short-term intensive training of HC breeders in molecular breeding.

**V. Engagement of USAID Field Mission(s):** During the main cowpea season July-September in 2016, the UC-R PI and Co-PI will make a field visit to HC Senegal to review and coordinate field based phenotyping activities. During this HC trip, we will arrange to visit the Senegal USAID mission. The mission visit will be made together with the respective Host Country PI and Co-PI plus senior NARS administrators where feasible, and will be used to inform the mission staff of our LIL cowpea modern breeding project goals and activities in the country and in the region. In Burkina Faso and Ghana, following field visits in 2014 to Burkina and 2015 to Ghana (planned), the UC-R team will assist the NARS PIs in developing project activity briefs for them to share directly with the US Mission staff to keep them informed and to solicit possible Mission buy-ins.

**VI. Partnering and Networking Activities:** We will work closely with other national and international cowpea breeders, including Drs. Ousmane Boukar and Christian Fatokun, Senior Scientists and Cowpea Breeders at IITA, Dr. Mohammed Ishiyaku of the IAR in Nigeria, Dr. Prince Addae, AATF, Nigeria, and Dr. Rogerio Chiulele, Eduardo Mondlane University, Maputo, in Mozambique. We will continue to work with national extension services, World Vision International, World Bank and other NGOs to extend new cowpea

technologies. Specifically in the Host Countries for this project, we will network with NGOs and farmers' cooperatives in Burkina Faso, Senegal, and Ghana. Although we do not have a formal seed systems objective in the project, the new cowpea varieties developed by the project will be fed into the NARS coordinated seed systems structure in each country. New varieties will be assured of entry and promotion in the seed systems. Exciting events are occurring to aid in this realization for seed multiplication and distribution to farmers. In Senegal, HC PI N. Cisse is working with World Bank on its recent $60M commitment to agricultural productivity of the cowpea seed system, while CORAF and AGRA with Foundation support are working to advance the seed systems in Burkina Faso, Ghana and neighboring countries. HC PIs I. Drabo and Benoit J. Batieno (INERA) and I. Atokple and F. Kusi (SARI) are involved in these efforts and can promote the introduction of the new CRSP and LIL cowpea varieties. This will be especially important for Objective 2 activities through which CRSP variety releases are in progress in Senegal and Burkina Faso. In Ghana the project is collaborating with the newly launched USAID cowpea dissemination project (Taking cowpeas to scale in West Africa). The LIL project team are actively involved in the planning, protocol preparation and implementation of the project. The five large white grain type cowpeas (at least 25 g /100 grains) developed by Dr. Cisse and other lines to be released by Burkina Faso will be helpful to the cowpea out-scaling project assuming access to the seeds in Ghana. The LIL project in Ghana is also collaborating with Promise project of CARE International which seeks to introduce improved quality cowpea varieties as well as IPM strategies for cowpea production to farmers.

**VII. Leveraging of CRSP Resources:** Other resources leveraged from current and future funded complementary cowpea research projects include the following:

California Dry Bean Advisory Board and its Blackeye Varietal Council (funds currently and typically set at $20,000 per year) funded for cowpea breeding in California. This is a continuing, long term research arrangement in support of the UC Riverside cowpea breeding program.

The CGIAR Generation Challenge Program (GCP) Tropical Legumes I Project Phase 2 funded from May 2010-April 2014 has been extended to November 2015. The cowpea component of this project is led by UC Riverside (Roberts and Close) and includes collaborative funded cowpea breeding and research with the cowpea breeding programs in Burkina Faso (with PI I. Drabo), Mozambique (PI R. Chiulele), Senegal (PI N. Cisse), and IITA-Nigeria (PI, O. Boukar). This project funded at $2.729M plus a $221,739 extension is applying cowpea genomic resources based on SNP genotyping for cowpea marker-assisted breeding. Use of the high throughput marker platform for major traits including insect pest, nematode and disease resistance, and drought and heat tolerance are being targeted in African breeding populations. A new project, Tropical Legumes III (Improving livelihoods for smallholder farmers: Enhanced grain productivity and production in sub-Saharan Africa and South Asia), funded by the Gates Foundation and administered by CGIAR-ICRISAT, with IITA leading the cowpea component, has been approved for funding for four years commencing in summer 2015. In the cowpea objective, UCR ($260,000) will contribute SNP genotyping work and guide its application in cowpea breeding, while INERA–Burkina Faso ($519,396) and SARI-Ghana ($319,271) will contribute trait discovery and breeding line development plus cowpea seed system development. These projects provide excellent leveraging for CRSP activities described here to be used for cowpea modern breeding. The projects also link us to the GCP-Integrated Breeding Platform project which is developing a breeder's

workflow system, which we are applying to the LIL project activities for data collection, analysis, interpretation and curation.

The project team plus Dr. O Boukar, IITA, Nigeria, led by Close and Roberts at UCR, were awarded $4,972,542 for five years starting September 2014 for the USAID Innovation Lab for Climate Resilient Cowpea. This project enables development of new cowpea genomic resources, particularly a 49,000-SNP Infinium iSelect genotyping platform developed during the last year. We are leveraging this advancement by applying it to our LIL project genotyping needs, thereby enhancing the quality and efficiency of the genotyping component.

UCR (Roberts and Close) has 2015 pending applications for cowpea funding with NSF/BREAD and USDA-NIFA for analysis and enhancement of the cowpea 8-parent MAGIC population and cowpea whole genome sequence.

The LIL funds proposed herein will also be leveraged with opportunity funds within the Host Countries via NGOs and national sources through presentation of the LIL effort and the associated opportunities for participatory funding.

AGRA is supporting multiple traits resistance breeding project at Kamboinse in Burkina Faso under Dr. Batieno.

Kirkhouse Trust supported Dr. Cisse at ISRA on molecular breeding for Striga resistance (July 2012 – June 2015; $90,000).

Kirkhouse Trust provided funding for SARI to improve the field resistance of five cowpea lines using MABC. These lines are current being evaluated in multilocation on-farm to gather the necessary data for their release. The five lines will serve as the recipients of the traits currently being screened under LIL. These traits include resistance/tolerance to Thrips, Striga, drought, heat, diseases as well as pyramiding the different sources of aphid resistance genes.

The Bt cowpea project being conducted by the Burkina Faso and Ghana HC teams is being funded by USAID via AATF.

**VIII. Timeline for Achievement of Milestones of Technical Progress:**

Please see completed "Milestones for Technical Progress" form for the workplan period.

**Training/Capacity Building Workplan for FY 2016**

**<u>Degree Training</u>:**

First and Other Given Names: Sassoum
Last Name: Lo
Citizenship: Senegal
Gender: Female
Training Institution: UC Riverside
Supervising CRSP PI: Close and Roberts, UC-R
Degree Program for training: PhD
Program Areas or Discipline: Cowpea genomics and breeding
If enrolled at a US university, will Trainee be a “Participant Trainee” as defined by

USAID: Yes
Host Country Institution to Benefit from Training: Senegal
Thesis Title/Research Area: Cowpea molecular breeding
Start Date: 03/2014
Projected Completion Date: 12/2018
Training status: Active, started degree program 03/2014
Type of CRSP Support: partial

**Degree Training:**

First and Other Given Names: Arsenio
Last Name: Ndeve
Citizenship: Mozambique
Gender: Male
Training Institution: UC Riverside
Supervising CRSP PI Roberts and Close, UC-R
Degree Program for training: PhD
Program Areas or Discipline: Plant Pathology, genetics and breeding
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID: Yes
Host Country Institution to Benefit from Training: Mozambique
Thesis Title/Research Area: Genomewide selection for disease and drought tolerance in SE African cowpeas
Start Date: 01/2012
Projected Completion Date: 12/2016
Training status: Active
Type of CRSP Support: partial

**Short-term Training:**

Type of training: Molecular breeding for young trainee breeders and NARS scientists
Description of training activity: As described under capacity building Objective 3, continuous short-term training will occur 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 will convene a training workshop in each project year, using a combination of training modules developed by the UC-R team and by the CGIAR GCP Integrated Breeding Platform program (IBP) which is using our tropical legumes project cowpea breeding population data for the training modules. The first two of these workshops were held in March 2014 and March 2015 at UCR. We will convene next year's training workshop immediately preceding the Pan-African Grain Legume and World Cowpea Conference.
Location: Livingston, Zambia
Duration 3 days
When will it occur? February 2016
Participants/Beneficiaries of Training Activity
Anticipated numbers of Beneficiaries (male and female): 12 (9 male, 3 female)
PI/Collaborator responsible for this training activity: Dr. Bao Lam Huynh, UC-R
List other funding sources that will be sought (if any): Training funds through USAID Climate Resilient Cowpea project will be leveraged to share costs.
Training justification: 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.

**Equipment** (costing >$5,000)**:** None requested during this period.