SASHA Phase 1 Final Report: Narrative for 1 July 2015 – 30 June 2016

I. Executive Summary. The perfect end to year 2 of SASHA Phase 2 (SASHA2) occurred on 28th June 2016, when Ambassador Kenneth Quinn announced at a ceremony at the U.S. Department of State, that CIP scientists Jan Low, Robert Mwanga, and Maria Andrade, along with Howdy Bouis of HarvestPlus, had been selected as the 2016 World Food Prize Co-Laureates. The award honored their efforts to improve nutrition through biofortification, highlighting in particular their work on orange-fleshed sweetpotato (OFSP). As of 8 July, more than 100 media outlets have carried the announcement. The actual awarding of the prize will be on 13 October in Des Moines, Iowa. The team warmly acknowledges the key role the Bill & Melinda Gates Foundation (BMGF) played in helping turn an innovative concept into reality and the catalytic role Foundation support plays in encouraging other donors to participate.

SASHA2 is building on the successes realized during SASHA1, with a strategic focus on adaptive research to break the remaining bottlenecks to unleashing the potential of sweetpotato to reduce undernutrition and food insecurity. In the first half of year 2, we integrated US $1.9 million of supplemental funds from BMGF into the core SASHA2 project, which enabled seven existing sub-grants with partners in national agricultural research systems (NARS) to be strengthened and two additional new sub-grants. Agreed sub-grant agreements (SGAs) for year 2 were finalized for 18 sub-grantees. The updated results tracker is provided in Appendix A. Of the 32 key milestones, 2 have been completed (6%), 24 are on track (75%), and 6 are behind schedule (19%). Appendix B provides the approved expenditures by CIP-HQ finance for the second year of SASHA2 (July 2015–June 2016).

RP1. Breeding. Population development is conducted at “Sweetpotato Support Platforms” (SSPs) in Uganda, Mozambique, and Ghana, with backstopping from CIP-HQ. Collaboration with 14 national partners ensures efficiency of breeding efforts, with an overall breeding goal in SASHA2 of 30 new improved varieties available by mid-2019. Specific breeding objectives are to (1) continue to improve sweetpotato population development in sub-Saharan Africa (SSA) linked with participatory varietal selection at national level; (2) breed for key biotic constraints in Africa; (3) breed OFSP populations for drought-prone regions in Africa; and (4) breed quality types of sweetpotato for urban markets. By the end of year 2, activities at CIP-HQ to exploit heterosis through reciprocal recurrent selection (RRS) were ahead of schedule. The achievements include the following: (1) The experimental heterosis populations work to determine the genetic gain for one RRS cycle using a heterosis exploiting breeding scheme (HEBS) has completed 80% of the RRS cycle. (2) Recombination of clones to form new less-sweet sweetpotato (LSSP) hybrid families was completed and multiplication of the hybrid seed is currently underway for planting the final step to prove HEBS for LSSP. (3) Recombination of parents to develop new gene pools for high Fe and Zn. (4) Implementation of testing the nearest neighbor design using the program “Agrobase” to adjust differences in performance of genotypes due to soil fertility in trials with large numbers of unreplicated entries. (5) Acquisition of equipment for fast-throughput methods to determine sucrose for LSSP and for sweetpotato virus disease (SPVD). (6) Five DArT markers were found to be associated with sweet potato chlorotic stunt virus (SPCSV) in two contrasting sets of clones (resistant set and susceptible, each comprising 12 clones). (7) Modified demonstration trials (MDTs) to measure genetic gain through comparison of yields of recently released varieties with older varieties, farmer varieties, and standard checks were introduced at HQ and planned at sites in SSA. In the regions, results from trials were recalculated to validate the potential to exploit heterosis under high virus pressure in Uganda, and demonstrated significant heterosis increment. This led to a decision to proceed to selecting strongly for virus resistance using RRS in partially inbred populations to be recombined to exploit heterosis. Second, seven new drought-tolerant cultivars (two OFSP; three dual purpose; two purple-fleshed) were released in Mozambique in February 2016, with details published in *HortScience*. In consultation with seed experts, breeders selected varieties designated as "100 Best Bet Sweetpotato Germplasm in SSA" for fingerprinting at the Biosciences eastern and central Africa (BecA), virus indexing, in-vitro tissue culture (TC), and in- vivo (screenhouse) conservation.

RP2. Weevil Resistance. Transgenic sweetpotatoes with sweetpotato-like *cry* genes were developed at the Applied Biotechnology Laboratory (ABL) in Peru; 63 transgenic events were tested for resistance against weevils. A more recent series of transgenic sweetpotatoes bearing high-constitutive-expression *cry* genes was produced at ABL in Peru and at the Donald Danforth Plant Science Center (DDPSC) in the US. To date, the accumulation of Cry proteins in sweetpotato storage roots appears challenging. Of a total of 132 transgenic events tested, we have identified 12 with apparent differences with the non-transgenic materials. This percentage of around 10% is within those observed for the engineered trait relying on transgene expression level. However, we have observed significant variation in infection efficiency from assay to assay, and we cannot rule out that these 12 events were simply poorly infected. Therefore, we will reconfirm these results with a new fresh batch of storage roots.

An RNAi strategy was developed to complement the *Bt* strategy. Our University of Ghent partners have identified three target genes that have given good mortality results for both weevil species in both soaking and artificial diets. Four hairpin gene constructs were delivered to CIP-HQ in early 2016 to start plant transformation. A total of 400 explants (meristems) and 4,800 leaves with petioles have been agro-infected with the first gene construct received (*snf7* of C. puncticollis). More transformation experiments are underway, and soon first regenerants from selective media will be tested for the presence of the transgenes. We believe that combining best weevil resistance genes from both strategies, *Bt* (Cry) and RNAi, represents our best option for controlling this devastating pest of the sweetpotato crop in SSA.

RP3. Seed Systems. By the end of year 2, national agricultural research institutes (NARIs) in 11 countries were testing models for the sustainable production of pre-basic seed. The biannual sweetpotato seed systems community of practice (CoP) meeting was held in December 2015, with 31 participants (including agricultural economists) from the institutions with SGAs. The meeting focused on a review of the pre-basic seed business plans and the use of a tool to estimate seed production requirements. Ten institutions submitted final business plans and a cross-country synthesis was prepared. Improved templates to capture real-time costs have been prepared and are in use by SGA partners in Nigeria, Ghana, Tanzania, and Kenya. Six of the 11 NARI SGA partners have reported income to their revolving fund, although the disbursement process and other operational modalities still need refinement. The Kenya Plant Health Inspectorate Service (KEPHIS-PQBS) hosted two, 5-day courses for NARI and private sector partners on "Tissue Culture (TC) Micro-propagation and Hardening" and "Virus

Identification and Testing." Pre- and post-training assessments were conducted and showed an improvement in knowledge. Participants returned to their institutions with action plans to improve TC and screenhouse practices and increase pre-basic seed production. The second bi-annual meeting of the broader CoP was held in May 2016 in Arusha, hosted by Crop BioScience and SRI-Kibaha. This meeting had the highest attendance and number of "non-SASHA-sponsored" participants to date.

Research continues on Triple S and net tunnel validation as well as comparing different small-scale irrigation techniques for vine multiplication and conservation. Training of trainers (ToT) courses on Triple S were implemented in Mozambique and Kenya. The sandponics experiment at KEPHIS varieties found that the optimal nitrogen level for five sweetpotato varieties is between 100 and 200 ppm; that all the sweetpotato varieties adapted well to the sandponics; and that a 10- plant density per pot performs better than 5 plants per pot.

Studies to test models for commercial basic seed enterprises (the "Missing Middle") have started in Tanzania and Ethiopia (supplemental funding for Objective 3.4). In Uganda (Agoro and Rakai), the isolation study and rice and sweetpotato seed rotation study have started and first season data collected. A rapid market assessment was conducted in May to understand seed system actors and seed flows in Northern Uganda and how basic seed produced on the Agoro irrigation scheme would be marketed.

The fourth iteration of the ClonDiag tool for virus detection was developed, and 250 arrays are being tested. Instruments needed for the redesign of loop-mediated isothermal amplification (LAMP) primers for sweetpotato viruses were purchased by CIP-HQ, enabling the development of a LAMP prototype. The current reagent components were found to have limited shelf life; however, in year 3, field testing will start in parallel with ongoing lab work to improve the shelf life of the reagents.

RP4. Postharvest and Nutritional Quality. The Natural Resources Institute (NRI) led a trial comparing the effect of different harvesting, soil removal methods, and packaging containers on the keeping quality of freshly harvested OFSP roots during a 14-day period in August 2015 in Homa Bay County, Kenya (Milestone report OBJ4MS1.1.C). Results suggest OFSP puree processors should encourage the farmers who source roots to manually wash the soil off the roots and air-dry them, and then avoid over-packing. They can transfer the roots to the factory, where they can be successfully stored for 2 weeks in sacks. NRI also oversaw the construction of a test (10 ton) curing and storage facility linked to a factory processing OFSP puree in Homa Bay County, Kenya; two storage trials were completed with mixed success. A third, using solar power only, is underway.

A study designed to investigate improved methods for fresh root transport in Ghana (Mid-term, Appendix F) produced recommended packaging containers and handling practices to reduce damage to fresh roots. An additional round of testing the "Double-S" method (using dry, cool sand) in simple mud boxes for storing roots for home consumption in drought-prone areas demonstrated good quality retention for 2 months, with the higher dry matter (DM) OFSP variety 'Nane' outperforming the lower DM OFSP variety 'Apomuden'.

Capacity to perform proximate analysis at the Food and Nutrition Evaluation Laboratory (FANEL) was put in place in year 2. This complements capabilities in microbial and carotenoid analysis. FANEL trained two PhD candidates and three master's students in food science and food safety as part of the capacity building in the region, and backstopped an OFSP puree factory food safety standards compliance. Four studies were done in year 2 to develop shelf-storable OFSP puree. To date, the best combination of preservatives is 1% sorbate, 1% benzoate, and 1% citric acid at a reasonable cost of 0.04 USD/kg. The product must be vacuum packed, and microbial load is reduced if it is heated *after* packing. This can extend the shelf life of OFSP puree for more than 1 month at room temperature with very little change in taste, smell, and flavor. However, the presence of sorbate extends the time needed for bread to proof, a challenge to be addressed in 2016. The *trans* beta-carotene content in the OFSP bread sold by Tusky's supermarket in Nairobi was determined to be 3.14 mg/100 g on a dry weight basis. A high-fiber OFSP, using washed but unpeeled roots, was developed. OFSP bread made from high-fiber puree was found to be acceptable to consumers.

RP5. Support Platforms, Knowledge Management, and Governance. A revised governance structure separating oversight for SASHA and the Sweetpotato for Profit and Health Initiative (SPHI) was announced at the SPHI annual meeting in September 2015. The new SASHA Project Advisory Committee (PAC) consists of five technical experts, the director general of CIP, and SASHA's BMGF program officer. The SPHI Steering Committee consists of nine organizations and five donors. Both advisory bodies attended the 6th Annual SPHI Technical Meeting on 30 September–2 October in Kigali, Rwanda, and held their first team meetings. The 2015 annual meeting was the largest meeting to date, with 106 participants from 14 SSA countries. It included a one-day exhibition with 29 booths open to the public and a demonstration of the drone technology for crop monitoring. The first annual "Sweetpotato in SSA Progress Report" was compiled and presented at the SPHI Steering Committee meeting. RP5 coordinated the preparation of 32 briefs, of which 14 concerned SASHA findings. A slide show highlighting SPHI achievements in 2015 was distributed to the CoP mailing list at the end of 2015.

Five successful CoP technical meetings were held between December 2015 and July 2016, with detailed minutes (OBJ5 Milestone reports: MS1.1.B; MS1.3.D; MS1.3.E; MS1.3F; MS1.3G) and presentations available. The Sweetpotato Knowledge Portal (SKP) was relaunched in February 2016, and a monthly E-Digest began in May 2016, circulated to all registered members. Significant progress was made in developing and testing standardized data modules, including Open Data Kit (ODK) software programs, for collecting data using smartphones or tablets with Android operating systems.