SASHA Phase 1 Final Report: Narrative for 1 July 2016 – 30 June 2017

I. Executive Summary. This has been an incredible year of recognition and celebration of the progress made in biofortification, and for vitamin A-rich orange-fleshed sweetpotato (OFSP) in particular. The awarding of the 2016 World Food Prize to three CIP scientists supported by SASHA (Jan Low, Maria Andrade, and Robert Mwanga) and Howdy Bouis, of HarvestPlus, on 13 October 2016, was a memorable event. A highlight of the associated Borlaug Dialogue was a panel discussion with the co-laureates moderated by former Bill & Melinda Gates Foundation (BMGF) CEO Jeff Raikes. There has been extensive media coverage of the award, and the CIP laureates involved in 18 speaking events due to the award. Two papers were published in *Global Food Security* in early 2017 summarizing progress in biofortification and OFSP development and dissemination. The team deeply appreciates the key role BMGF played in helping turn an innovative concept into reality and the catalytic role it has played in getting other donors to support the going-to-scale effort.

SASHA2 is building on the successes realized during SASHA1, with a strategic focus on adaptive research to break the remaining bottlenecks to unleash the potential of sweetpotato to reduce undernutrition and food insecurity. Substantial progress was made in year 3. The only scientific disappointment being that the *Bt* approach for developing a weevil-resistant sweetpotato has not succeeded after several attempts to increase expression of the transformed genes. Agreed subgrant agreements (SGAs) for year 3 were finalized for 17 subgrantees. The updated results tracker is provided in Appendix A. Of the 32 key milestones, 3 have been completed (9%), 19 are on track (59%), and 10 are behind schedule (31%). Most of those behind schedule are due to postharvest experiments and seed system activities taking longer than anticipated. Appendix B provides the detailed expenditures by CIP-HQ finance for year 3 of SASHA2 (July 2016–June 2017).

Research Program (RP)1. 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 the 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 3, significant progress had been made in genetic gains experiments, improved techniques for collecting and analyzing breeding data, and seed distribution from the Uganda and Mozambique population development programs to national agricultural research systems (NARS) partners. Experimental heterosis populations work continues to be on track. One clone (MUSG15052-2) was identified in the Mozambique high-iron (Fe) breeding effort that, at 4.4 mg/100 g, exceeded the minimum target level of 2.4 mg/100 g (25% of RDI for a child 4–8 years old) set by the breeding program. In Ghana, 21 diverse genotypes were assessed for sensory attributes related to poundability and fry quality, and their starch properties were assessed using a rapid visco-analyzer.

Different approaches were used at CIP-HQ, Mozambique, and Ghana to assess genetic gains based on breeding program progress. At CIP-HQ, over the last two decades leading up to 2014, yield storage root gain at 90 days ranged from 0.18 to 0.34 t/ha per year; at 120 days, this increased to 0.36–0.58 t/ha per year. In Ghana, a storage root yield gain of 0.27 t/ha per year occurred between 1999 and 2012. In Mozambique, total storage root yield increased by a range of 0.287–0.303 t/ha annually and foliage yield by 0.084–0.104 t/ha annually between 2000 and 2016.

The need for continued breeding at a subregional level was verified by testing the 2011 successful Mozambique released varieties in Ghana and Uganda. The Mozambican varieties succumbed to viruses in both settings. Although 13 national breeding programs are now using the accelerated

breeding scheme (ABS) approach, it is of great concern that the Alliance for a Green Revolution in Africa (AGRA) issued no new breeding grants for sweetpotato during the past 2 years. Only one African-bred variety was released this year. To date, during phase 2, the population development program in Uganda has provided 361,366 seeds to 8 NARIs; the one in Mozambique distributed 128,570 seeds to 12 NARIs.

A new, highly interactive data analysis platform (HIDAP) was released in January 2017, and breeders trained on its use for 1.5 days at the annual SpeedBreeders meeting in May 2017. The breeding team led or were co-authors on six breeding-related articles this year, reflecting the finalization of breeding cycles and several experiments. A draft chapter on sweetpotato breeding for a book is at the proofing stage.

RP2. Weevil Resistance. Over the last eight years, transgenic sweetpotatoes with sweetpotato-like *cry* genes and with high-expresser *cry* genes were developed at the Applied Biotechnology Laboratory (ABL) in Peru and the Donald Danforth Plant Science Center (DDPSC) in the USA as a strategy to confer resistance to weevils. Well over 150 transgenic events were produced of which a total of 132 transgenic events were tested in a bio-assay using storage roots infested with 10 weevil females. We identified 12 with apparent differences with the nontransgenic materials in adult emergence. Storage roots from 10 of them were shipped from DDPSC to Biosciences eastern and central Africa (BecA) and tested for resistance against weevils. Six showed no significant difference in adult emergence compared with the untransformed storage roots. The remaining 4 presented various degrees of differences such as small number or delayed adult emergence. New repetition of the bioassay on several of these positive events did not confirm previous observation. These results, and those obtained previously, indicate that the amount of *Cry* protein accumulating in the storage roots is not high enough to confer significant level of resistance to the sweetpotato weevils.

The RNAi genes targeting weevil genes have been used in agro-infection of leaf with petiole and embryogenic calli from the sweetpotato variety 'Jonathan'. Up to now, six regenerants from infected leaf-with petiole were confirmed to be polymerase chain reaction (PCR) positive, whereas more regenerants from infected embryogenic calli are being isolated. Next steps, high expressers, storage root production, and bio-assays will take place when new sources of funding become available.

RP3. Seed Systems. All but one of the milestones in this diverse RP are on track. Concerning **technology improvement**, **net tunnel** validation research was completed in Tanzania and Ethiopia and a scientific paper is in preparation. A revised net tunnel brochure is ready for layout and printing. In Rakai District, Uganda (high virus pressure area), preliminary data from the ongoing trial comparing use of the standard net tunnel and mini-screenhouse, indicate that per unit area, for 'Ejumula' and 'Kabode' (but not 'NASPOT 11'), the highest number of cuttings is produced from the net tunnel compared with the mini-screenhouse. Comparative cost-benefit analysis is in progress. **Triple S**[1] activities are now under way in eight countries through collaboration with other projects. In Tigray, on-station validation experiments have shown that the roots survived more than 7.5 months of dry period, extending the potential of Triple S to fit up to 9 months of dry period. The findings from the validation process across different countries are now being consolidated into training of trainers (ToT) and farmer resource materials. In Tanzania, the on-station and on-farm study to assess different **types of irrigation equipment and schedules** is nearing completion. Overall, experimental plots performed better than farmer plots with highest number of vines produced under 10 kPa[2]. Data collection for the second **sandponics** experiment was completed.

[1] Triple S is the method in which roots, not cuttings from vines, are the main sources of seed. Designed for areas with at least 4 months of dry season, the roots are **S**tored in **S**and during the dry season and then **S**prouted when the roots are planted out in a protected garden 6–8 weeks before the rains start.

[2] kPa refers to kilopascal, which is a unit of measurement, in this case for soil water content; 0 kPa = very wet and 100 kPa = very dry.

Technical production capacity for pre-basic and basic sweetpotato seed production is being consolidated across the 11 countries. Screenhouse or mobile net tunnel production capacity ranges from 120–145 m^2 to 1,670 m^2. Year 3 estimated total production is 2,029,074 pre-basic and 7,103,890 basic three-node cuttings. NARI colleagues continue to increase multiplication rates and reduce costs through further experimentation. Fully updated cost structures (based on real-time cost data collection) have been completed for the Kenya Plant Health Inspection Service (KEPHIS) and the Crops Research Institute (CRI) of Ghana. The revised cost structures have been used to determine the break-even price, discuss mark-up and margins, and develop pricing strategies. The business plan templates have now been adapted to include production targets for different categories of seed, unit production cost, total recurrent production costs, proposed prices, current availability of revolving funds, and projected revenue. The filled-in templates will drive the SGA modifications for the remaining 18 months of this component.

The study protocol for the **pre-basic/early generation seed (PBS/EGS) validation study** was prepared and piloted with KEPHIS and the Rwanda Agricultural Board (RAB) using a participatory peer-to-peer methodology. The objective is to document pre-basic seed production models and to assess the changes in capacities and the level of institutionalization of the business plans in the NARIs. An improved methodology, drawing on the pilot experience, will be implemented with the remaining 11 institutions between August 2017 and June 2018.

Research to test and document models for medium- to large-scale basic seed production (the "missing middle") is ongoing in Uganda, Tanzania, and Ethiopia. Training was provided in seed agronomy (including inspection standards), enterprise, and marketing skills in all three countries. Business plans have been prepared and implementation started in northern Uganda and Ethiopia.

Efforts to encourage the **development and institutionalization of protocols for seed standards** for sweetpotato are proceeding well. In Tanzania, the seed standards for sweetpotato, potato, and cassava (pre-basic, basic, certified 1, and certified 2) were officially gazetted on 20 January 2017, joining Ethiopia, Kenya, and Zambia. The process continues in another seven other countries. As countries roll out the implementation of seed standards and inspection procedures, bottlenecks are emerging as evidenced in Ethiopia.

In the Agoro Irrigation Scheme in Uganda, the second and third cycles of the **rice-sweetpotato rotation experiment** were harvested in October 2016 and May 2017, respectively. There was a significant difference in net profits for both rice and sweetpotato in the rotation versus the control. All rice varieties had significantly higher net profits in the rotation than in the control. In contrast, for sweetpotato, there was no significant difference in net profits in the rotation and the control.

Research on determining the potential importance of sweetpotato **begomoviruses** continued. A field survey has been conducted in five out of the six regions that produce sweetpotato in Kenya. Symptoms associated with begomoviruses were present in most of the fields visited. Three-hundred samples have been tested by PCR to confirm presence of begomoviruses. Yield impact will be accessed by field testing in July–December 2017, at the Kenya Agriculture and Livestock Research Organization (KALRO) Kiboko and Marigat.

Diagnostic tools. A fourth iteration of ClonDiag was designed by FERA with improved performance and newly discovered viruses. The following viruses can be detected: CMV, SPCSV, SPV2, SPFMV, SPVG, SPVZ, SPMMV, SPCV, SPVCV, SPC6V, SPPV, and TSV. To analyze the result from the arrays, a smartphone app was developed and finalized into a stable version. A successful validation experiment was performed on 25 samples at CIP-KEPHIS. This obtained a good signal quality and the correct

viruses were identified. Detailed analysis comparing ClonDiag results from Lima and KEPHIS versus the standard indexing practice is being undertaken.

Progress has also been made in the development of a thermostabilized loop mediated isothermal amplification (LAMP) test for sweet potato virus disease (SPVD). It is in a ready-to-use form, user-friendly disease detection technique and requires no cold chain. We are currently optimizing parameters such as effect of lyophilization on PCR reagents, accessing heat stability of lyophilized PCR mixture, and improvement of heat stability. LAMP assays were previously developed for eight sweetpotato viruses but, owing to performance issues, we decided to focus on the main viruses sweet potato chlorotic fleck virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), and begomoviruses. Testing will continue through the remainder of 2017. We envisage that the portable LAMP device will be used in seed systems diagnostic and regulatory functions across SSA countries.

At the June 2016 breeder's meeting, 116 "best bet" varieties and elite materials at SSPs were selected to be fully characterized through field phenotyping and fingerprinting. To date, 86 best bet varieties out of the 116 have been submitted. Twenty-five varieties were established at the KALRO Kiboko field station for phenotypic trait characterization at the end of 2016; another 25 were established in May 2017. Twenty-five varieties were sampled for genotyping by simple sequence repeat markers at the BecA–International Livestock Research Institute (ILRI) Hub. All varieties will undergo virus cleaning by meristem tip culture and thermotherapy, be virus indexed, and conserved in-vitro tissue culture (TC) and in-vivo screenhouses. Core amounts of pathogen-tested cuttings will be maintained at KEPHIS to facilitate prompt response for specific varietal requests.

RP4. Postharvest and Nutritional Quality. The Natural Resources Institute (NRI) leads fresh root storage trials in Kenya comparing a solar-powered unit to an electrical-powered unit with generator backup. The fourth storage trial conducted from December 2016 to April 2017 was successful, with over 70% of the root weight of all treatments able to be processed into purée after 4 months' storage at 20–23°C in the solar-powered store. The variety and vertical height position of the crate in the store room had the most significant effect on the quality of the stored roots, followed by whether the store room was solar or mains powered. Full details of the storage trial are given in the Milestone report "Storing fresh sweetpotato roots long-term to reduce purée supply chain risks" (OBJ4MS4.1.B) and the design of the evaporative cooling storage facility in OBJ4MS4.1.C. EcoTech, a South African firm, did initial testing of the use of the "coolbot" to force AC units to store at lower temperatures. Results indicate that 12°C can be obtained. A model using a 20-ft container was built starting in May 2017 and will be field tested in Mozambique in year 4.

During year 3, the RP4 team achieved its goal of a shelf-storable OFSP purée that could store at room temperature for 4 months using locally available preservatives. Trials in Kenya showed that OFSP purée treated with 1% citric acid, 0.2–0.5% potassium sorbate, and 0.2–0.5% sodium benzoate, together with vacuum packing, can extend the shelf-life of OFSP purée by 3–6 months under ambient conditions. However, the preservative potassium sorbate affects yeast during dough development used in bread, lengthening dough proofing time and decreasing bread volume. This required further recipe development to mitigate these two negative side effects. Another breakthrough was the introduction of a stronger puréeing machine that could manage the sweetpotato peel in addition to the flesh. Combined with improved root-washing protocols, this enabled the production of *high-fiber* sweetpotato purée. Seven experiments regarding OFSP purée or OFSP purée bread were conducted during this period.

The development of shelf-storable purée has been done in collaboration with another project, SUSTAIN-Kenya, and the Organi Ltd factory in Ringa, Nyanza Province, Kenya. Working with this private sector company to establish a value chain linking OFSP farmers to the factory-making purée to the

bakery division of Tusky's supermarket chain in Nairobi, has meant that the cost and logistic aspects of purée development have been captured as part of the purée product development process. This helps to ensure that the OFSP purée can be an economically viable, convenient product. The development of this value chain has been captured in an article published by *Open Agriculture* entitled "From Lab to Life: Making orange-fleshed sweetpotato purée a commercial reality" (OBJ4MS4.3.A).

Capacity to perform beta-carotene and other high performance liquid chromatography (HPLC)-based analysis (e.g., vitamin C) at the Food and Nutrition Evaluation Laboratory (FANEL) increased after another used HPLC, obtained from CIP-HQ, was installed in September 2016. During year 3, FANEL conducted 2,302 analyses of beta-carotene, including for cassava and maize samples from HarvestPlus and OFSP products from the University of Development Studies (UDS) (Ghana). FANEL evaluated the beta-carotene content and proximate analysis for four bakery products used in Rwanda. In addition, it assessed the beta-carotene content and proximate analysis for OFSP breads prepared by substituting wheat flour with 0%, 20%, 30%, 40%, and 50% OFSP purée. Testing for bacteria, molds, and yeast continued to expand, confirming that Organi Ltd is compliant in food safety procedures and as part of shelf-life analyses of OFSP-processed products. FANEL developed a cost-recovery business plan to help assure its long-term sustainability as a regional lab of excellence in nutrient composition and food safety services. This will come into effect in August 2017. To date, FANEL has hosted eight graduate students working on OFSP and other roots, tubers, and banana (RTB) crops.

RP5. Support Platforms, Knowledge Management, and Governance. Twenty-one different organizations attended the 1.5-day 7th Annual SPHI technical meetings (Milestone report OBJ5MS1.2.E). The associated SASHA Project Advisory Committee (PAC) and SPHI Steering Committee (SCC) meetings were held on 7–8 October 2016, in Addis Ababa, Ethiopia. The SPHI meeting, with 94 participants, was aligned with two key events also held in Addis Ababa: the 10th Triennial African Potato Association (APA) Conference, held on 9–12 October (with 300 participants), and a cocktail celebrating CIP's 45th anniversary as an organization and the awarding of the World Food Prize for biofortification. Not surprisingly, the theme of the meeting was *A Time of Celebration.* Annual briefs were prepared: 15 for SASHA project updates and 24 for other sweetpotato projects, plus the second Status of Sweetpotato in SSA report.

The 16th Sweetpotato SpeedBreeders Annual Meeting was held on 15–18 May 2017, at the Grand Legacy Hotel in Kigali, Rwanda. It highlighted the introduction of the new program, HIDAP, for designing, collecting, analyzing, and reporting trial data (Milestone report: OBJ5MS1.1.C). Four other community of practice (CoP) technical meetings were held between December 2016 and July 2017, with detailed minutes (OBJ5 Milestone reports MS1.3.H, MS1.3.I, MS1.3.J, and MS1.3.K) and presentations available on the Sweetpotato Knowledge Portal (SKP). The 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) and CSPro software programs, for collecting data using smartphones or tablets with Android operating systems. A manual was prepared for nine core monitoring and evaluation (M&E) modules for indicators needed by most dissemination projects (OBJ5MS1.4.E) and training provided in its use at the monitoring, learning, and evaluation (MLE) CoP meeting.