Year 4 Annual Technical Report: Narrative for 1 July 2012–30 June 2013

Executive Summary

The Sweetpotato Action for Security and Health in Africa (SASHA) is a five-year project that seeks to directly improve the food security of at least 155,000 Sub-Saharan Africa (SSA) families by exploiting the untapped potential of sweetpotato and to create the conditions for going to scale. This requires (1) transforming sweetpotato breeding, (2) developing innovative seed systems, (3) strengthening partners' capacities, and (4) understanding how to link these components to market and food-based nutritional interventions while assuring gender equity. The project has completed its fourth year. This report covers progress made through 3o June 2013. Note that during this reporting period, a two person external evaluation team visited all major SASHA field sites from 20 October – 2 November 2012, with a visit to SASHA research in Peru 3-7 December 2012. In March 2013, SASHA PMT members and some key partners attended a four day convening at the Bill & Melinda Gates Foundation. In preparation for that meeting, an on-line review of major SASHA partners was conducted to receive feedback on the role of CIP as a lead organization and "two-pagers" of key areas of research for Phase 2 prepared. After reviewing the results from the external evaluation and progress-to-date, BMGF invited the SASHA team to develop a concept note for SASHA Phase 2 by 31st July 2013.

Overall, most components from four of the five research programs (RP) are on track based on their original milestones. The exception is RP2 (weevil resistant sweetpotato), which redesigned their program in year 3 and the new strategy is advancing well. Of the 177 currently approved milestones[1], 106 have been completely or almost achieved (60%), 27 (15%) are on track for their expected completion dates, 28 (16%) are behind schedule and 16 (9%) have not yet started. The majority of "behind schedule" milestones are for the breeding program (longer to multiply material for the trials than anticipated; additional validation work) and the animal feed program (delays by two students in finishing their theses). Explanations for any delays are provided in the main text and detailed milestone table in Appendix B. Appendix A provides an updated log frame of outputs. Highlights for this period are summarized below. OBx.x refers to relevant objective numbers and MSx.x to milestone numbers.

The project lost one of its important partners in RP2 this year, Dr. Jesse Machuka of Kenyatta University, who passed away on 9th May 2013. Dr. Machuka was a champion of biotechnology. Through his research laboratory and research program, he trained more than 40 graduate students.

RP1: Breeding and Varietal Improvement. The overall objective of RP1 is to develop improved breeding methods and establish efficient population improvement programs at a sub-regional level in SSA, linked with participatory varietal selection at the national level. At CIP HQ, preliminary results are now available for a large experiment that critically evaluates the efficiency of breeding using controlled crosses versus polycrosses. Research on systematic exploitation of heterosis in sweetpotato breeding continued, with a) most seed production completed in Uganda for evaluation of heterosis in stressful environments in Mozambique and Uganda, and b) crossing underway at CIP HQ to look at the potential to

[1] Note that 3 additional milestones were added to RP4 when the COVA study was added in May 2012; and many milestones from RP2 were dropped and 3 new ones added when the sub-program was re-designed.

apply reciprocal recurrent selection in sweetpotato, and c) to exploit heterosis in small populations high in micronutrients or low in sugars. Resistance to sweetpotato virus disease (SPVD) in germplasm introduced from CIP HQ to Uganda has held up under field conditions at levels comparable to the most resistant Ugandan clones. Progenies from crosses and self-pollinations among genotypes resistant to SPVD are already being evaluated in Uganda and present the possibility of significantly increasing the frequency of SPVD resistant genotypes in breeding populations in the near future. In Mozambique, a large number of advanced clones selected for drought tolerance using accelerated breeding are ready for trialing on-station and on-farm in year 5. In Ghana, breeding of less sweet sweetpotato for West Africa progressed well with improving efficiency of hybridization, and the introduction of new sources of low sugar germplasm from CIP HQ. Consumer taste tests of genotypes in advanced trials indicated good consumer acceptance of emerging genotypes. Strengthening of the community of practice in target countries continued, including a two week sweetpotato breeders' course held in August 2012 at the University of Ghent, focused on learning molecular methods, the annual breeders' course in April 2013 held in Kigali, Rwanda, nine in-country trainings on the use of *CloneSelector*, and the distribution of germplasm from SSPs to national program partners. Sammy Agili completed his PhD in breeding at the Jomo Kenyatta University.

RP2: Breeding Weevil-Resistant Sweetpotato (WRSP). This research program aims at the development of weevil resistant (WR) varieties of sweetpotato using a transgenic approach. Three WR genes have been introduced into sweetpotato varieties and produced close to a hundred transformed events (transgenic plants) with *Cry* genes. Thirty-one of them, from the variety Jewel, were not found to display activity against weevils. Year 4 activities aim to verify proper expression of functional proteins. Of the first batch of transgenic events from the variety 'Jewel' we have retested efficacy against weevils for one transgenic event and finalized the confined field trial using 3 transgenic events per single gene construct. At NaCRRI, one event with accumulation of the ET33-34 protein in the range of the LC_{50} of ET33/ET34 did not show mortality of the African weevil, *Cylas puncticollis*, which could indicate that the assembly of these two Cry proteins as a fusion protein inactivates them. In Puerto Rico, we have conducted the first confined field trial using the best transgenic events from Jewel with the objective to identify sub-lethal activity. Nine transgenic events were tested. One transgenic event expressing the toxin *cry3Ca1* gene under the control of the sporamin promoter displayed sub-lethal activity against the weevil *Cylas formicarius*. This will be re-confirmed in Puerto Rico in year 5.

Understanding where the problem of the lack of efficacy lies continued to be investigated. We have extracted total proteins from several transgenic events and confirmed by Western blotting that Cry7Aa1, Cry3Ca1, and ET33-34 are expressed as protein of the expected size and quantities as measured by DAS-ELISA. For ET33-34, experiments are underway. However, the proof that the proteins expressed in sweetpotato roots are functional is still lacking. Progress has been made on the verification of the correct expression of the inserted *cry* genes. Cry proteins are accumulating with its expected size at levels below or equal to the LC_{50} of the corresponding native Cry protein. The discovery of sub lethal activity of one transgenic event expressing the Cry3Ca1 toxin, if confirmed, points at low accumulation of the Cry proteins in storage roots as the cause of lack of efficacy. A transient expression assay to test new gene constructs indicates higher Cry protein production of Cry3Ca1 than Cry7Aa1. Because both proteins appear to interact with at least one common weevil midgut receptor,

we may focus in the future transformation experiments on gene constructs expressing the Cry3Ca1 protoxin. Screening for efficacy of 64 transgenic events, transformation with new gene constructs, and a non-protein base resistance strategy are the main activities going on and expected to be completed beyond project termination.

RP3: Sustainable Seed Systems. Richard Gibson, our NRI collaborator, finished his leadership of objective 1 under SASHA during the first half of year 4. Findings from an experiment he led in Uganda have provided evidence of reversion from sweetpotato feathery mottle virus (SPFMV) in certain varieties. Sam Namanda obtained his PhD in November 2012 based on the studies carried out under objective 1; his Triple S research work was accepted for publication by the Journal of Crop Improvement. MARI scientists completed the virus degeneration study of 5 varieties across 10 sites through 4 field generations in the Lake Zone of Tanzania. Virus infection did not seem to increase steadily over the generations but declined or at least was held in check, probably due to good rogueing practices. These results reinforce the importance of breeding for virus resistance.

The dissemination phase of Marando Bora (objective 2) was completed in June 2012 with a slightly revised estimate of 111,912 beneficiaries reached of which 74% estimated to be women. Polista and Kabode were the best performing varieties under Marando Bora. However, under the project conditions, there did not appear to be significant yield differences between the cleaned up material and farmers' own negatively selected material. Initial results from the pilot testing the use of sweetpotato Quality Declared Planting Material (QDPM) guidelines show that it is possible to implement a community based inspection scheme, but the feasibility of meeting the FAO tolerance levels will vary by variety, agro-ecology and management practice. The endline survey was designed, including recommendations provided by the BMGF gender-strengthening effort led by the International Center for Research on Women. The field work for the end-line impact survey for Marando Bora was implemented between January and March 2013 among 730 households; data entry completed by end of May 2013, and analysis and write-up are on-going at the present time.

A brochure was produced on why to use a net tunnel to prevent virus infections and how to construct one. Net tunnel research was expanded to Rwanda, with one net tunnel being established with each of the 20 farmer's groups in Super Foods project, as well as 2 tunnels with SINA Enterprises. This material is part of a study to look at the potential for commercializing quality vine sales in a market-oriented project.

In December 2012, we started the process of integrating the tissue culture information system at KEPHIS-PQS into a diagnostic system. KEPHIS-PQS and CIP-Nairobi staff were trained on the use of bar coding technologies for managing genetic resources; both *in vitro* and *in vivo*. Further analysis of siRNA sequencing data identified several new viruses. The same samples used for siRNA sequencing were used to test the second iteration ClonDiag array, the results of which together the sequence data were used to develop the third iteration tube-array. The third iteration array is now ready and will be sent to CIP and MARI for additional testing and validation. A working prototype of a smartphone app to record and analyze tube-array results has been developed, but is still being further improved to assure reliable function under various circumstances. This app which is HTML5-based and works across operating systems, will omit the need for laboratories to acquire specialized array-readers and software to analyze results.

RP4: Effective Delivery Systems. Mama SASHA, the integrated agriculture and nutrition intervention that links orange-fleshed sweetpotato (OFSP) access to ante-natal services for pregnant women, received additional funding in May 2012 to conduct a longitudinal nested cohort study (referred to as **COVA**) among 500 pregnant women. All staff members for the COVA component were recruited and trained, and data collection began in December 2012. Since the implementation of wave 2 (March 2011 to May 2013), Mama SASHA monitoring data indicate that 7,417 pairs of vouchers have been issued to 5,359 pregnant and lactating women, far exceeding the project's goal of reaching 900 women; 3,837 pairs of these vouchers have been redeemed (approximately 52%) for vines. **COVA** staff members have completed the enrolment of 505 participants (the 1st visit) and 69% of the mothers have also completed their second visit; 311 of the participants have given birth so far. The **Rwanda Super Foods Project**, which seeks to establish sweetpotato processed product value chains, officially launched the Golden Power Biscuit on 9th November 2012, and it is being sold in sachets and tubes (50 gm and 100 gm units) by SINA Enterprises in its 8 shops around the country. The media campaign was launched, with over 51 events, including 5 TV programs, 4 print news articles, 26 radio programs (11 different stations), 12 online media articles and participation in several agricultural fairs. In September 2012, the team started a monthly newsletter and 10 issues have now been published in English and Kinyarwanda. OFSP product sales by the factory have increased with the selling two mandazi (doughnut) types, the Golden Power Biscuits, and bread with gross revenue reaching US $160,373 this year, a 150% increase over last year. Results from the **sweetpotato value chain study in Nigeria** were presented at a major Sweetpotato Stakeholder consultation in Abuja, Nigeria in July 2012 at which the Minister of Agriculture and Rural Development committed to investing in orange-fleshed sweetpotato in 2013. Under the **Animal Feed** component, an additional masters' student finished and two continued to write up their findings to write up their findings on dual purpose varietal selection and on the pig feeding trial with sweetpotato silage. Additional work was undertaken on improved silage recipes, combining different levels of sweetpotato foliage with Napier grass or maize stover.

RP5: Management and Support Platforms (SSP). The SASHA project successfully organized and held its third Annual Technical Meeting and the SPHI Executive Steering Committee meeting on 11-14 September 2012 in Nairobi, Kenya. A half-day exhibition highlighting SPHI products was a new addition to the annual meeting, during which a new five minute video on the Mama SASHA project was launched. The fifth round of Sweetpotato Support Platform (SSP) meetings were conducted in East and Central Africa and in Southern Africa, with funds for the West Africa meeting used to sponsor West African scientists to attend the 16th Triennial Symposium of the International Society of Root and Tuber Crops meeting held in Abeokuta, Nigeria and sponsor a special section on SASHA findings. The 6th SSP meeting was held in West Africa in February 2013, with funds for the other SSP meetings used to hold 9 country level trainings of technicians and breeders on CloneSelector 3.0 and to hold topic-specific SASHA Phase 2 consultation meetings on breeding, pig feeding, seed and production systems, and post-harvest research.