GT4SP Quarterly Project Report
Investigator: International Potato Center (CIP)

Objective 1. Development of the core genomic and genetic resources for sweetpotato improvement

Objective 1.A Genome sequencing

CIP contributed to a manuscript for the genome reference led by the Boyce Thompson Institute (BTI) and Michigan State University (MSU).

Objective 1.B. Transcriptome profiling

During the reporting period CIP was involved in data analysis and manuscript development for the abiotic stress RNAseq experiments, together with MSU. This work is ongoing.

Objective 1.C. Development of diploid mapping populations for high-density single nucleotide (SNP) genome sequence anchoring and quantitative trait loci (QTL) mapping

There is no activity to report for this objective during this reporting period.

Objective 2. Establishment of a genotyping-by-sequencing (GBS)-based SNP genotyping, bioinformatics, and analytical environment for hexaploid sweetpotato

Objective 2.A. GBS-based genotyping platform development at North Carolina State University (NCSU)

There are no activities to report on developing GBS platform with NCSU during this reporting period.

Objective 2.B. Software for calling genotypes and phased haplotypes from raw 6x GBS data, coupled with software to construct 6x linkage maps from genotypes called from GBS data

There are no activities to report on testing new software from NCSU and Univ. of Queensland during this reporting period.

Objective 2.D. Databases for genotypic, phenotypic, and QTL information compatible with the CGIAR Generation Challenge Program Integrated Breeding Platform

Following the successful completion of the previous activities by the end of January, to be able to better serve the breeding community represented by the Sweetpotato Action for Security and Health in Africa (SASHA) project, the new team undertook to round-trip HIDAP and SweetPotatoBase to make the work flows more efficient. The specific activities agreed upon are:

1. ***Integration between FieldbookApp data and HIDAP–SweetPotatoBase***
    - Develop a R plug-in module in HIDAP–SweetPotatoBase and a user interface for FieldbookApp files (XLSX, others)
    - Map data formats of the FieldbookApp
    - Develop the R converter script from FieldbookApp format to HIDAP–SweetPotatoBase
    - Print labels
2. ***Data quality module for SweetPotatoBase fieldbooks***
    - Deployment of the HIDAP Data Quality module in SweetPotatoBase
    - Internal cross-checking variables names (traits) of FieldbookApp field data according to crop ontology standards

- Check metadata information of SweetPotatoBase fieldbooks.

3. Fieldbook Registry module in HIDAP–SweetPotatoBase

- Add a user login module using Sweetpotato LDAP with Brapi
- Develop R scripts for uploading fieldbooks between HIDAP and SweetPotatoBase
- Check BrAPI functionalities for uploading fieldbooks
- Develop a R user interface for uploading fieldbooks

4. Testing and documentation of the HIDAP–SweetPotatoBase integration

- Check of data validation from BTI Team
- Test and validate the connection with SweetpotatoBase
- Generate examples and use cases for the HIDAP–SweetPotatoBase integration
- Give maintenance of HIDAP-R packages in the BTI servers

These activities are ongoing and the team is working closely with BTI.

Objective 3. Population development, multilocation phenotyping, and marker-assisted breeding (MAB) studies

Objective 3.A. Selective or target phenotyping and QTL analysis of two diploid *I. trifida* populations

Phenotyping of the M9 x M19 population is already complete. QTL analysis and manuscript development are ongoing between CIP and NCSU; all data are analyzed. Given contributions to several manuscripts at the same time, this work is still ongoing.

Objective 3.B. Genotyping, multilocation phenotyping, and QTL analyses of previously developed 6x New Kawogo x Beauregard and Beauregard x Tanzania (BT) mapping populations

Phenotyping of the BT population in Peru is complete. Preliminary data analysis for phenotypic data is done. Multilocation yield and yield components data have been provided to NCSU to develop QTL mapping software and leading to a joint manuscript led by NCSU to which CIP is contributing. Phenotypic data analysis for quality traits was completed in March 2018. In collaboration with NCSU bioinformatics team, QTL mapping for these traits was done in March 2018. Manuscript preparation, led by CIP, is ongoing.

In Ghana, the last field experiment for the BT population was planted in Nyankpala in September 2017. Drought and well-watered conditions were imposed and the experiment has been harvested. Screenhouse evaluations are being carried out of root system development under drought and non-drought conditions in 50 BxT genotypes with contrasting drought response under field trials. The experiment is ongoing at Fumesua and will be completed in the next quarter. Work was conducted primarily by WACCI student, Obaiya Utoblo. Trial data are being analyzed, and QTL mapping is anticipated with assistance of CIP–HQ and NCSU partners. In Ghana, the BT population is being maintained in screenhouse for possible use in other linkage studies—for example, for virus resistance or quality traits.

In Uganda, the last experiments of the BT populations, two seasons 2016/2017, were done at three sites (Namulonge, Serere, and Kachwekano); the last site (Kachwekano) was harvested in December 2017. Data are being analyzed. All phenotypic data will be used for QTL mapping once the software is complete.

Objective 3.C. Genomic selection (GS) studies and new population development for GS and marker-assisted genetic gain studies

Objective 3.C.1. Preliminary GS studies

DNA extraction of the 3,000 genotypes of the P2 and P4 populations are complete. However, genotyping will not be done unless additional resources are identified to carry out GS work in Peru.

No activities to report during the reporting period

Objective 3.C.2. Population development for implementation of MAB and GS in sweetpotato-breeding programs in sub-Saharan Africa (SSA)

The last batch of plantlets of the Mwanga diversity panel (MDP) population has been shipped during the last quarter of 2017 to the National Crops Resources Research Institute (NaCRRI) in Uganda from Biosciences eastern and central Africa (BecA) in Kenya. All together, we have 62 out of 64 families with 30 genotypes and above established in vitro at the tissue culture facility of the BecA/ILRI (International Livestock Research Institute) hub in Nairobi. In vivo in Uganda, 48 families have the required 30 genotypes; 10 families have 29 genotypes; 2 families have 28 genotypes; and the rest have 27, 25, 12, and 5 genotypes. The gaps in number will be filled promptly. In Namulonge, the population has been planted in net tunnels (one tunnel/family) for multiplication of vines ready for planting. The team elaborated the planning of multiplication for March/April 2018 planting of field experiments. Plans were also developed for the phenotyping experiment (in Namulonge, Kachwekano, Serere, and Abi) two seasons and 10 plants/genotype, plus 16 parents and two common varieties as checks. These were 'Ejumula', susceptible to sweet potato virus disease (SPVD), and 'NASPOT 11', resistant to SPVD in single replication in Westcott design. SPVD, Alternaria blight, weevil resistance (by NaCRRI), and quality traits will be the key traits for phenotyping. A few samples had discrepancies related to the labeling on the shipment list and screenhouse. These will be sampled and fingerprinted using single sequence repeat (SSR) markers and compared with the corresponding original genotype at BecA to verify that they are true to type before being immediately established in the field. Leaf tissues of the 16 parents of this population were collected from the net tunnels. DNA was extracted at NaCRRI and sent to NCSU for targeted GBS analysis prior to receiving DNA samples of their progeny. DNA extraction is ongoing (currently 20 families are done). These samples will be sent to NCSU for targeted GBS after the parents' analysis is completed. This is a GBS that offers efficiency for discovering, validating, and screening genetic variants using a highly targeted approach. Targeted GBS approaches detect novel variants in specific genome regions, and provide a lower cost alternative for some genotyping applications. Deep sequencing of targeted regions also helps to characterize rare variants.

Objective 4. Traditional and web-based training and capacity development efforts to incorporate MAB tools into sweetpotato-breeding programs in Africa

Objective 4.A. Engage key SSA stakeholders by organizing regional, in-country, and web-based workshops on the potential of genomics-based breeding methods in sweetpotato

1. **Long-term trainings:**

   We have four trainees under the ABCF program funded by BecA (minimum of 6 months with possible 1-year extension):

   - Gaspard Nihorimbere (researcher/Institut des Sciences Agronomique du Burundi [ISABU] - wheat and sweetpotato) reported on 15 April 2017.

Project tittle: "Population diversity and GWAS of sweetpotato varieties in Burundi"
Progress: Has finished his 11 months of the ABCF fellowship

- Key findings
  - Genetic diversity and relatedness of 172 sweetpotato genotypes known
  - 6 SNPs markers associated with beta-carotene content identified
  - 11 SNPs markers associated with weevil resistance identified
- Future work to be done on the validation of the SNPs and conversion to KASP markers for MAS of the traits
- Findings of this study will be used to make informed decision on the parents to use in Burundi sweetpotato-breeding program and germplasm conservation. Markers will be validated and used for MAS in SSA.

- Joanne Adero (research associate, NaCRRI–Uganda) reported on 1 July 2017.

  Project tittle: "Molecular variability of sweetpotato viruses in Uganda"

  Progress: Fellowship contract is requested to be extended by 3 months (January–March) to allow time for data analysis and sequencing of the remaining libraries
  - Paired-end sequencing for 24 out of 48 RNA samples was done using the Illumina MiSeq 2000 instrument. The other 24 samples are still awaiting sequencing.
  - Preliminary results: About 2m reads (all samples) were obtained and were of good quality. These were then mapped to the sweetpotato reference genome (*I. trifida*). A *de novo* assembly was done for the unmapped reads and the contigs and scaffolds were blasted onto NCBI to obtain virus signatures. The virus signatures will be blasted onto the sweetpotato virome database to assess genetic diversity of the Ugandan samples versus all samples in Africa uploaded to date. New virus sequences will be uploaded in the sweetpotato virome database.
  - Results and discussions will be made available with the breeding teams addressing SPVD and Uganda regulatory body for management of the disease.
- Eunice Wainaina (Ministry of Agriculture, Livestock and Fisheries–Kenya) reported on 10 July 2017.

  Project tittle: "Carotenoid gene markers for sweetpotato; applications in genetic diversity and marker assisted breeding for increased β-carotene content

  Progress:
  - Fellowship contract was extended by 3 months to allow time for validation of SSR markers, molecular data generation and analysis, nutritional analysis, and correlation studies between SSR markers and nutritional content.
  - We targeted developing at least 20 SSR markers from carotenoid pathway genes. We have managed to get 8 SSR markers from seven genes: phytoene synthase, carotenoid isomerase, zeaxanthin epoxidase, NCED3, carotenoid cleavage, geranyl geranyl pyrophosphate (GGPP), and isopentyl diphosphate. The 8 markers mined from the *I. trifida* genome were designed and validated insilco and wetlab using four ß-carotene phenotypes (cream for 'Tanzania', white for 'Mugande', orange for 199062.1, and deep orange for 'Bella').
  - Polymerase chain reactions for 96 DNA samples (83 from the 100 best-bet varieties and 13 varieties from farmers' fields around Kiambu County, Kenya) have been run, 6

markers from the genes isopentyl pyrophosphate isomerase, GGPP, and carotenoid cleavage deoxygenase carotenoid isomerase, zeaxanthin epoxidase, and NCED3 showed specificity. Capillary fragment analysis (ABI 3730) and data generation (Genemapper) is ongoing.

All genotypes that had been planted at the University of Nairobi–Kabete for storage root formation for nutrition analysis have been harvested. Nutritional analysis for ß-carotene, dry matter, and sugars at the Food and Nutritional Evaluation Laboratory–BecA laboratory is ongoing.

Results from this work will be shared with the SASHA team, in sweetpotato-breeding meetings, and workshops.

- Bararyenya Astere (ISABU, Burundi) reported in February 2018.

  Project tittle: "Genome wide association studies (GWAS) and QTL analysis for continuous storage root formation and bulking traits in sweetpotato".

  Progress: Bararyenya has been facing challenges (low DNA yields) with DNA extraction from lyophilized leaves using a Zymo kit. Since NaCRRI has been empowered to do molecular work through the project capacity building and training, he has gone back to extract DNA from fresh leave samples. So far he has extracted 96 out of 300 samples and will bring the DNA for DArTseq and GWAS analysis at IGSS-BecA lab.

It is mandatory for every ABCF fellow to write a manuscript and publish work done in a peer-reviewed journal; Mercy Kitavi is a co-author on all published papers. The capacity-building efforts of the GT4SP project will be acknowledged.

2. Short-term trainings

- There is no activity to communicate during the reporting period. BecA is going through a transition period as several important grants end; activities in this category may be much affected.

3. Workshops

- The annual SASHA/GT4SP breeders meeting is been planned for 5–8 June 2018, with the proposed theme of "Breeder tools for data management, efficiency and analysis." GT4SP will probably have 1-day presentation on the use of genomic tools in sweetpotato breeding and ½-day training on SweetPotatoBase and HIDAP.

4. Webinars:

- Planning is underway for the first SweetPotatoBase and HIDAP introduction and training webinar with the BTI team. Owing to technical issues, the webinars have been moved to the end of April. A follow-up on the third week in May (tentative dates to be communicated) will target the breeders in the national programs in SSA in preparation for the annual breeders meeting.

5. Regional breeders support:

- During the SASHA project meeting, the CIP breeders agreed to send preserved leaf tissues of parental germplasm to BecA to be genotyped with 66 SSR markers for heterosis study. A CIP global objective for sweetpotato breeding and objective is to look at heterosis-combining ability and GG of parents from different gene pools. The breeders (Ghana and Mozambique)

were supported and provided with standard operating procedures for leaf tissue preservation by lyophilization developed as one of the GT4SP training materials for molecular work in sweetpotato. Both breeding programs failed on the first attempt to properly lyophilize leaf tissues, and therefore the quality of tissues has degraded. Currently, leaf tissues of 64 genotypes from Ghana have been sent and DNA extracted. We are awaiting leaf tissues from 134 genotypes from Mozambique. We realize that the breeders still have a challenge sampling and preserving leaf samples for DNA extraction. Therefore more training is needed in this area.

Objective 5. Project management and communication processes to ensure project success

During the reporting period, the project was represented by CIP at the Grand Challenges by the Gates Foundation in October. D. Gemenet visited NSCU breeding programs and also had discussions with the bioinformatics team in October. CIP was also involved in representing the project during the Sweetpotato for Health Initiative meeting in Tanzania in October. The CIP data management group and CIP breeders met at NCSU, together with the BTI team, to further discuss data management and analytics for breeders. CIP was involved in planning the sweetpotato and yam genomics workshop held at the Plant and Animal Genomes conference in San Diego in January 2018, during which some of the results from the project were communicated. CIP participated and presented during PAG in January 2018. CIP participated in monthly executive meetings, and contributed to a concept note for Phase II in March 2018.