

Progress Narrative

Use this form to provide updates to your foundation program officer regarding progress made toward achieving your project's stated outputs and outcomes.

The Progress Narrative must be submitted in Word, as PDFs will not be accepted.

Progress and Results

1. Progress Details

Provide information regarding the current period's progress toward achieving the investment outputs and outcomes as well as the work planned or anticipated for the next period. In addition, submit the Results Tracker with actual results as requested.

BREEDING DIVISION

The progress made on Year 1 Breeding Division project milestones, as well as the work planned for Year 2 is shown below, organized by breeding-focused project outputs. The institutes referenced below are: the International Institute of Tropical Agriculture (IITA), Nigeria; the National Root Crop Research Institute (NRCRI), Nigeria; the National Crops Resources Institute (NaCRRI), Uganda; the Tanzania Agricultural Research Institute (TARI); the Brazilian Agricultural Research Corporation (EMBRAPA) in and the International Center for Tropical Agriculture in Colombia (CIAT).

Germplasm exchange: Collaboration and exchange of indexed germplasm with Latin American breeding programs enable the introgression of genetic variability into African breeding populations.

Importation of the Cassava Brown Streak Disease (CBSD) immune cassava clones from South America (CIAT) will form an integral part in CBSD resistance cassava breeding in Africa especially in the regions where the disease is ravaging. Five cassava clones were introduced in Tanzania in 2017 in batch one and eighteen were introduced in batch two in 2018. In a preliminary screening of batch 1 clones, only one showed CBSD symptoms. All the clones in batch 1 have been planted in four locations with high CBSD pressure. In addition, they have been planted in a crossing block to introgress resistance genes into African elite cassava clones for CBSD resistance.

From CIAT, four clones (two with high carotenoids and putative CBSD-immune) were shipped to the USA for their eventual release to be planted in the crossing nursery in Hawaii. There are 16 remaining clones ready for shipment to Maryland as soon as APHIS informs CIAT. In vitro plantlets of >150 new genotypes produced, indexed and ready for shipment upon receiving the import permits. A total of 5984 botanical seeds from controlled pollinations (full-sib families) produced: 28 crosses of high-carotene (HTCC)/low cyanogenic potential (LHCN) x resistance to CMD (RCMD) (743 seeds); 10 crosses of HTCC/poor culinary quality x RCMD (435 seeds); 82 crosses of HTCC/good culinary quality x RCMD (3,002 seeds); and 86 crosses of maximum HTCC x RCMD (1,604 seeds). A total of 40,044 botanical seeds from open pollination nurseries has been produced (42 half-sib families involving HTCC and RCMD). Of particular relevance in the work to combine resistance to whiteflies and CMD. CIAT produced hundreds of F1 genotypes, which were then screened for reaction to white flies. Only 27 F1-genotypes were selected and crosses among them were made to produce a pseudo-F2 generation. A total of 170 full-sib families were obtained from crosses among the 27 genotypes (9,000 seeds), also 13 S1 families (214 seeds) and 11 "F3" full-sib families (417 seeds) from few F2 genotypes that had been obtained years ago were produced. DNA from the 27 F1 genotypes was extracted and shipped so that CIAT can identify which of those carry the CMD2 source of resistance. The material is ready for shipment as soon as the respective import permits are issued.

EMBRAPA shipped 99 *in vitro* parents (*M. esculenta*) from their training population to Leibniz Institute DSMZ for phytosanitary and diagnostic services. For wild species, which are not under the FAO Treaty, the Brazilian Genetic Heritage Council (CGen) has revised the legal procedures for germplasm exchange and a new form of Material Transfer Term was released in 2019. Therefore, the NextGen project was registered in CGen and around 3,500 seeds of 11 *Manihot* species will be shipped to African partners in 2019. For the next period, EMBRAPA plans to achieve the following: Material Transfer Term signed and 3,500 botanical seeds of 11 wild cassava species shipped to Africa; standard material agreement signed and 97 GS-C1 parents of *M. esculenta* transferred to Leibniz Institute DSMZ; for preemptive breeding for CMD and CBSD resistance, transfer to EMBRAPA of 18 CIAT clones with potential CBSD resistance.

Please see Appendix 1 Part 1A for more details on germplasm exchange at CIAT and EMBRAPA.

Disease resistance: Varieties with durable CBSD resistance developed in East Africa; pre-emptive breeding of CBSD-resistant varieties for West Africa.

This is a new breeding outcome that we are proposing, as efforts have been focused on this important work, and it will provide new alleles for more durable CBSD resistance.

A subset of CIAT Genebank Core Set of cassava germplasm found to be resistant to cassava brown streak disease (CBSD) was distributed to African breeding partners in Nigeria, Uganda and Tanzania.

Germplasm derived from hybridization between IITA and CIAT genotypes in Hawaii were evaluated in a seedling nursery in 2018 (see further information below, under “Intercontinental hybridization initiative”). Using both phenotype and marker-assisted selection, we advanced 800 genotypes to a clonal evaluation trial in Ikenne for 2018 – 2019 field season. The CMD2-linked marker in the CIAT x IITA seedling nursery predicted resistance and susceptibility with an accuracy rate of 73%. The 27% error rate resulted from either false negative (17.2%, i.e. susceptible while predicted to be resistant) or false positive cases (9.76, i.e. resistant while predicted to be susceptible).

As part of their efforts towards developing varieties that specifically integrate resistance to cassava brown streak disease (CBSD) with other user-preferred traits, NRCRI exchanged a total of 3,058 botanical seeds derived from 120 families with the Ugandan project partner (NaCRRRI) for purpose of testing for CBSD resistant alleles in our gene pool. In addition, we equally made progress in various stages of variety development pipeline as indicated by the advancing and phenotyping of 1,029 clones in the CET stage, 170 clones in the AYT stage and 43 clones in the UYT stage. For generation of new population, a total of 18,568 recombinant seeds were generated for C3 population.

At NaCRRRI, a total of 864 clones sourced from West Africa (specifically from IITA) were evaluated. Assessments were made for both defensive and agronomic traits notably: cassava mosaic disease (CMD), cassava brown streak disease (CBSD), cassava green mite (CGM), root dry matter content (DMC), harvest index (HI), fresh root yield/plant (FRY), and beta carotene content. (Datasets associated with this trial are located at: <https://www.cassavabase.org/breeders/trial/4527>.) 248 cassava clones sourced from CIAT were introduced and evaluated at Namulonge. Assessments were made for traits, notably CMD, CBSD, CGM, DMC, HI and FRY. (Datasets associated with this trial are located at: <https://www.cassavabase.org/breeders/trial/4384>.) A total of 1,980 seedlings generated from different family combinations were introduced from NRCRI (Nigeria) and evaluated in Uganda. Accordingly, both CBSD and CMD resistance assessments have been made at three and six months after planting. (Datasets associated with this trial can be accessed at: <https://www.cassavabase.org/breeders/trial/5312>.)

TARI undertook evaluation of cassava breeding lines from coastal lowlands in the Lake Victoria area: A number of cassava breeding lines tolerant to CBSD were developed in the eastern coastal lowland of Tanzania where CBSD infection have been endemic ever since the disease was reported in 1936. Due to CBSD crisis in the Lake Victoria region, it was decided that all the best breeding lines from the eastern coastal lowland be assembled and evaluated in the Lake region. A total of 108 cassava breeding lines were assembled and bulked at TARI Ilonga under irrigation in August 2018. The estimated number of cuttings that have been produced by March 2019 ranged from 12 to 400 cuttings. The number of breeding lines with high estimated number of cuttings will be put in screening CBSD trials in the Lake Victoria area; trials will be established in November 2019.

Intercontinental hybridization initiative

NextGen has been collaborating with the University of Hawaii Manoa, Hilo, Hawaii, USA and the USDA Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center Tropical Plant Genetic Resources and Disease Research Unit Hilo, Hawaii to hybridize African and Latin American cassava genotypes for the purpose of introducing CMD resistance into Latin American derived germplasm in a virus free environment. The outcome of this collaboration will allow intercontinental germplasm exchange between Asia, Latin America and Africa. In 2015, we were able to obtain approval for import of botanical seed from selected genotypes from IITA in Africa and CIAT in Colombia to initiate a trial hybridization exercise. In 2016, seedling progenies were grown in Hilo. Most seedling individuals flowered and it was possible to harvest both full sib cross seed and open pollinated seed representing IITA and CIAT derived parents. The first seed was shipped to IITA in December 2017. Additionally, CIAT has been attempting to obtain an import permit so the seed can be imported to Colombia to serve as new sources of CMD resistance. As of May 2019, the import to ship the second batch of seed from Hawaii to Africa has been received with seed packaged and shipment expected on 30 May 2019. The process of obtaining the import permit for seed shipment to Colombia, Thailand and Vietnam is continuing. In 2015, CMD was first reported in Asia. Since that time there has been increased interest to import CMD resistant germplasm to Asia. A list of open-pollinated seed has been prepared for export to Vietnam and Thailand when import permits can be received.

Sixty-eight selected seedlings from the December 2016 Hilo seedling nursery were planted in a clonal crossing block in January 2017. The crossing block used alternating rows of CIAT and IITA clones to assist with performing controlled crosses or distribution of pollen for open pollinated seed. The clones were established in two blocks corresponding to yellow root biofortified germplasm and white root, high starch populations. All of the African genotypes carry CMD resistance. A full season of pollinations was completed by March 2018. A total of 2,677 full sib family seeds have been produced from 151 unique cross combinations representing 66 of the 68 selected clones. Open pollinated seed was collected from 47 of the 68 clones with 15,113 seeds collected from 24 IITA-derived clones and 7,756 seeds collected from 23 CIAT-derived clones. The harvested seed has been prepared for export to NextGen partner in Africa through IITA, CIAT in Colombia, and National Programs in Vietnam and Thailand. In April 2019, the import permit from IITA was received and

the seed has been packaged for shipment to IITA pending with the shipment scheduled for 30 May 2019 following preparation of the phytosanitary certificate. Seed lists have been prepared for shipments to African programs through IITA in Nigeria, CIAT in Colombia, Thailand and Vietnam.

Please see Appendix 1 Part 1B for more details on breeding for CBSD resistance at NaCRRRI, TARI, and the Hawaii initiative.

Crosses: Suitable parents identified, crossed, and advanced in accelerated pipeline for release.

Completed 2017-2018 field trials at IITA: IITA conducted 40 field trials involving clones from successive generations from genomic selection cycles and stages of field testing for the 2017-2018 season. These trials consisting of a total of 5,286 plots were established in eight locations (Ibadan, Ikenne, Mokwa, Ubiaja, Onne, Otobi, Umudike and Ago-Owu). Advanced selections from GS cycle 1 (29 entries and 5 standard checks) were tested for second season of Uniform Yield Trials (UYT) at six locations. Likewise, first season testing of advanced selections (62 entries and 5 checks) from GS cycle 2 was carried out in three locations.

Ongoing 2018 – 2019 field trials at IITA: Currently, IITA has 50 field trials with a total of 7,669 plots for the 2018-2019 season. These trials are scattered across 11 locations in Nigeria. The number of locations were expanded compared to last season in order to ensure adequate testing of our clones in the target population of environments. The added locations were Zaria, representing the Northern Guinea Savannah, and Abuja, representing Southern Guinea Savanna. The first pre-release evaluations were planted across six IITA-managed sites and an equal number established by NRCRI in the National Collaborative Research Program (NCRP) trial. It includes 8 clones from GS cycle 1, 6 clones from NRCRI breeding program and four checks.

Single site and multi-environment trial analyses for experiments harvested in the 2018 season have been completed. Heritability values from individual trials were above 0.5 for most traits across three stages of phenotyping i.e. PYT, AYT and UYT, an indication of good trial quality data (Figure 1).

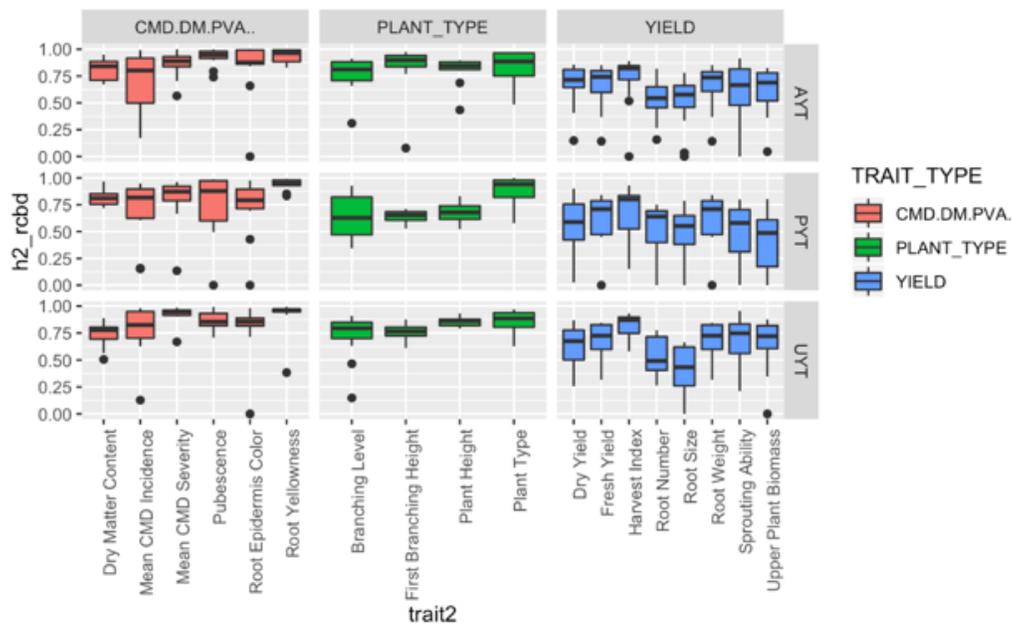


Figure 1. Boxplots showing the range of heritabilities obtained from 18 traits across 36 replicated trials.

Genotype-by-environment analyses carried out using 34 clones in Uniform Yield Trials from GS cycle 1 planted in 10 environments enabled us to determine the magnitude of GxE for key traits, the correlation between environments and the best performing clones for different environment groups. Results of this analysis will be used to rationalize selection of testing sites to maximize genetic gain and eliminate redundant testing in correlated environments. In the next period, clone advancements through the variety release pipeline will be prioritized. Specifically, GS C1 clones will be taken to on-farm trials; C2 clones will be in their first National Performance Trials; C3 will go into their first Uniform Yield Trials. The early stage testing will also be continued.

At NRCRI, 18,568 recombinant seeds were derived from biparental crosses of C2a clones for generation of C3 population. 8,076 seedlings were established in the seedling nursery for C2b population. 1,029 clones were advanced and evaluated under CET; 170 clones advanced and evaluated under AYT; and 43 clones advanced and evaluated under UYT.

Four key results were achieved at NaCRRRI: a) Recombinant seeds generated from hybridizations between elite Latin America (LA), West Africa (WA), and East African (EA) cassava clones; b) Different cross combinations undertaken among 50 elite pVAC cassava clones to generate a C2 population; c) Field evaluation data generated from 67 (elite white-fleshed) and 25 (elite pVAC) clones

established in advanced yield trials at four sites in Uganda; and d) Field evaluation data generated from C2 white-fleshed seedlings established at Namulonge.

At TARI, one of the major activities undertaken during this period was generation of cassava botanical seeds from genetic crosses involving cassava parents that were selected from the Cassava Training population. 54 genotypes selected as parents from the previous season training population (2018/2019) were raised in a crossing block that were established at TARI Maruku, which was the main crossing block. In addition, Namikonga, AR 40-6, Mkumba, Kiroba, TZ130, Kizimbani, Orela, F10-30R, Kipusa were added to the list of parents based on their reaction to CBSD. Genetic crosses among the parents to produce F1 for C1 evaluation were conducted based on the clustering of the parents following genetic diversity analysis of the parents prior to pollination. These cross combinations produced a total of 65,445 full sib cassava botanical seeds and 67,957 half sib cassava seeds at TARI Maruku. A total of 4,313 botanical seeds (F1s) resulting from different cross combinations of all parents from clusters 1 to 4 were obtained at TARI Kibaha. A total of 2,332 and 3,379 half sib cassava botanical seeds were collected at TARI Kibaha and Ukiriguru, respectively. In addition, part of this seed lot of half sibs, 26,560 seeds have been planted at Ilonga to generate Cycle 1 cassava seedling nursery.

1st cycle of genomic selection (GS-C1) at EMBRAPA: The GEBVs from 2,028 seedlings were estimated based on the G-BLUP, and then 100 seedlings were selected from the GS-C1 based on the selection index: $\frac{1}{2} \text{FRY} + \frac{1}{2} \text{DMC} + \frac{1}{2} \text{StY}$, where FRY, DMC, and StY are the GEBVs for fresh root yield, dry matter in the roots, and starch yield, respectively. From the 100 GS-C1 parents, 97 were established in the field-crossing block. Other 739 GS-C1 clones were selected based on its phenotypic performance and planted in CET in augmented block design with 20 blocks and 6 checks.

2nd cycle of genomic selection (GS-C2) at EMBRAPA: The GS-C1 parents were divided into four clusters aiming to maximize the obtaining of contrasting GS-C2 progenies. The crosses began in March 2019 and the seeds from this cycle will be available in September-October 2019.

For the next period, EMBRAPA plans GS-C1: 30-50 parents crossed; 739 clones in CET, 150 selected for advancement to AYT. GS-C2: 4000 seedlings; at least 150 Brazilian genotypes evaluated for drought tolerance in semiarid area with focus on root and shoot yield; at least 180 Brazilian genotypes evaluated for resistance to cassava bacterial blight, anthracnose and super-elongation in hotspot areas in Central-South Brazil; and GWAS to discover natural variation contributing to stress tolerance and shoot disease resistance in cassava.

Please see Appendix 1 Part 1C for more details on crosses and field trials at IITA, NaCRRI, TARI, and EMBRAPA.

MAS (screening): Identified trait-linked markers used for early selections.

At IITA, a proof-of-concept genotyping trial using large seedling nurseries planted in 2018 was carried out using the trait-linked markers for CMD, provitamin A and dry matter content. The accuracy rate of the CMD2-linked marker in correctly predicting resistance and susceptibility within the IITA GS population was 86%. The false negative and false positive rates were 11.3% and 2.9%, respectively. On the other hand, the accuracy rate in the CIAT x IITA hybridization population was 73%. These findings are expected to guide the usage of the markers in the breeding pipelines.

In implementing and marker-assisted selection at NRCRI, 363 elite clones with Latin American introgressed genes were screened with CMD2 and DMC markers and these are being analyzed. It will be reported in the next period.

From NaCRRI, a total of 846 leaf samples comprising of Latin American, West African and East African clones under evaluation at Namulonge were submitted to Intertek for SNP genotyping using candidate markers for DMC, CMD, CGM and beta-carotene. A total of 9 plates have been submitted. Plans for moving forward are to validate markers associated with CMD, CBSD and beta carotene in both early and late selection stage trials. We await data for the nine submitted plates.

1,653 samples from TARI were sent to Intertek for DNA extraction and then for DaRTSeqL genotyping. Part of these genotypes are the 54 parents that will be genotyped for quality check against C1 seedlings.

1,783 cassava clones from Brazil were genotyped by KASP markers developed for CMD resistance and beta-carotene content by the IITA team. The SNP effects analysis for both traits in the cassava Brazilian gene pool is ongoing. Validation of 5-marker panel for marker-assisted selection is planned for the next period, to select Brazilian germplasm with high beta-carotene.

Please see Appendix 1 Part 1D for more details on MAS implementation at IITA, CIAT, and EMBRAPA.

GS predictions: Genomic Estimated Breeding Values and Genomic Estimated Total Genetic Value used to select clone for crosses and advancements in the breeding pipelines.

In 2018 IITA established a large seedling nursery consisting of 22,420 individuals from controlled crosses between 216 parents from GS cycle 3 selected from GEBVs and multi-environment phenotypes. We selected 5,000 seedlings and advanced same to clonal evaluation trial and genotyping using DaRTSeq to rebuild the IITA training population for genomic predictions. A third of the seedlings earmarked for genotyping used to plant partially replicated CET while the remaining two thirds were planted in Ibadan for stem multiplication. The

2019 seedling nursery in Ibadan was established using 26,509 individuals from 538 families involving 242 unique parents carried out in Ubiaja in the 2018 crossing season. Phenotypic evaluation is ongoing.

Single site analyses for 18 selected traits by fitting linear mixed model in all trials (n=36) that were planted using a randomized complete block design with sub-blocks. These trials were mainly PYTs with two replicates, AYT with three replicates and UYT with three replicates. Further, 22 of the replicated trials had row and column plot coordinate information which allowed to fit a spatial model to correct for spatial trends in the field. Different models were considered: RCBD (rep and genotype effect alone, ROW-COL (row, column and genotype effect), RCBD-Spatial (row, column, rep and genotype effect). Heritability was estimated on entry-mean basis. GGE and Finlay Wilkinson models were fitted to explore GXE interactions in the multi-environment trials. GXE interaction was further partitioned into genotype main effect, environment main effect and a sensitivity estimate.

NRCRI employed GBS to genotype the C2a population due to the delay in finalizing the procedure for DArTseq platform. The GEBVs derived were used to select the best 25 parents for the C3a population. In addition, 95 C1b clones selected based on GEBVs were advanced and evaluated in the AYT. In the next period, NRCRI plans to implement GS using the DArTseq genotyping platform with C2a and C2b populations. Best clones will be selected and advanced to the next stage from the Seedling nursery, CET, AYT and UYT trials. NRCRI is leading the nationally coordinated research project (NCRP), collaborating with IITA and other stakeholders, which is the official pre-release variety trials in Nigeria. Currently, the first season trial is ongoing in 10 locations in Nigeria and 8 GS-derived clones from IITA's C1 cycle and 6 non-GS derived clones from NRCRI and 4 check varieties, each site with 3 replications.

At NaCRRRI, advanced yield trials (AYT) were established at five sites: Namulonge (central region), Busia (eastern), Serere (eastern), Arua (west Nile), and Kasese (southwestern). C₁ white-fleshed clones were established in trials having 36 plants/plot, while C₀ pVAC clones were established in trials with 25 plants/plot. All trials were replicated twice at each site. Of these sites, only three sites had good crop establishment, and thus were used for data collection on plant growth, CBSD, and CMD resistance assessments. The data that has so far been collected for the 63 C₁ and 24 C₀ clones across the three locations are respectively uploaded in Cassavabase: <https://www.cassavabase.org/folder/4441> and <https://www.cassavabase.org/folder/4441>. In the next period, crossing nurseries will be established for selected C₁ pVAC and C₂ white-fleshed clones, as well as seedling nurseries for progeny generated by crossing LA, WA and EA clones. Clonal evaluation trials of selections made from WA, LA and NRCRI germplasm will be replicated. AYT and UYT will be established for both pVAC and white-fleshed selections.

At TARI, 54 parents were selected from Cycle 0 in the previous season. 18 clones were selected from the training population for advancement to AYT at Ukiriguru, and 84 clones at Kibaha.

Please see Appendix 1 Part 1E for more details on GS predictions at IITA, NRCRI, and TARI.

SAH: Semi-autotrophic hydroponics (SAH) used to increase cassava clone propagation efficiency.

Currently, there are 2,997 plantlets of 10 NextGen clones in the SAH laboratory of IITA. They are: TMS13F1053P0010, TMS13F1053P0015, TMS13F1343P0022, TMS13F1343P0002, TMS13F1153P0001, TMS13F1160P0004, TMS13F1176P0012, TMS13F1307P0016, TMS13F2110P0008, and TMS13F1343P0044.

The NextGen clones from NRCRI currently in the pre-release multilocal trial will be initiated into SAH. At NaCRRRI, no multiplication has been done using SAH-generated materials, but there is a plan to undertake SAH using virus-free plantlets generated through a thermotherapy process (virus-free plantlets are yet to be generated). NaCRRRI has obtained the license for an SAH lab and have began a capacity building exercise for relevant staff members.

In this period, no multiplication was done at NaCRRRI using materials generated by SAH. We plan to undertake SAH using virus-free plantlets generated through a thermotherapy process. Virus-free plantlets are yet to be generated.

Variety release (selection): Nominated varieties advanced for release in years 2, 3, and 5.

Although there were no release targets for Year 1, work has been done toward this milestone in this period. The first pre-release evaluation been planted across six IITA-managed sites and an equal number established by NRCRI in a National Collaborative National Performance (NCRP) trial. It includes 8 clones from GS cycle 1, 6 clones from NRCRI and four checks. Second season evaluation of GS cycle 2 UYTs (i.e. UYT36setA and UYT36setB) is underway across eight locations. Together with NRCRI, IITA has nominated eight clones to be included in national collaborative trials implemented together with NRCRI in 10 locations. These trials will be harvested between June and July 2019.

Root processing into garri and fufu: To gain better understanding of the phenotypic and genetic variation for garri and fufu – the two most important food products from cassava in Nigeria, IITA processed 20 kg of storage roots from six Uniform Yield Trials of advanced selections from the first two cycles of genomic selection: *GS cycle 1 UYT34*, *GS cycle 2 UYT36 set A*, *GS cycle 2 UYT36 set B*. Phenotypic analysis of these traits uncovered good heritability values across trials, indicating a strong genetic variance component. Garri yield (H^2 0.61 to 0.86) had slightly higher heritability compared to fufu yield (0.31 to 0.77). Garri, fufu and dry matter content were correlated with Pearson's r values between 0.46 to 0.72 indicating that dry matter content is a good proxy for indirectly selecting for these traits. As expected, fufu fiber content and fufu products were found to have a large negative correlation of -0.64.

At NaCRRRI, TRICOT trials have been established in three regions with around 240 farmers. And at TARI, 100 elite breeding lines have been assembled from the coastal lowlands, multiplied at TARI Ilonga and are ready for AYT evaluation in Lake Zone towards variety release. Further information on TRICOT trials is included below under Survey Division results, and in the Appendix 1 Part 3A.

Please see Appendix 1 Part 1F for more details on pre-release evaluations at IITA and NRCRI.

Other breeding activities:

The IITA team from Ibadan took part in harvesting and phenotyping of field trials in Namulonge and Sendusu, Uganda. In Namulonge, they carried out joint evaluation of a clonal trial derived from seeds shipped from Ibadan and assessed CBSD root necrosis, yield components, dry matter and carotenoid content. In Sendusu, we participated in joint harvest and phenotyping of clonal and preliminary yield trials.

The joint NextGen (IITA) and Cassava Sink Source Phase 2 proposal has been approved by the Bill and Melinda Gates Foundation. The collaborative project seeks to carry out biochemical and physiological analysis of diverse clones from IITA to better understand genetic, biochemical and physiological components that underpin variation in important cassava traits, especially yield and starch productivity.

Collaboration with Excellence in Breeding (EiB)

In Year 1, NextGen Cassava began to work closely together with the EiB's multi-faceted modules. We see alignment and appropriate interaction with EiB as critical to the success and sustainability of NextGen Cassava. A strong partnership between EiB and NextGen is a win-win and we are working to make the connections at the coordination level so that the entire EiB modules 1-5 were properly engaged synergistically with NextGen Cassava. The project seeks to be a model of what the EiB desires to achieve, even in a public sector breeding program working with poor-resourced NARS in Africa. If we can achieve this, both EiB and NextGen will reap many mutual benefits that will help actualize their joint mission. The following is a summary of the EiB/NextGen interactions that have occurred in the current reporting period.

Module 1 (Product design and management), led by George Kotch, has really engaged with the project team leadership and members at Cornell and the others in Africa (at IITA and the different NARS). We have made a lot of progress together in the areas of Product Profiles development and stage-gating of the breeding processes.

We held a workshop of NextGen Cassava breeders (NARS in Africa) and IITA cassava breeders in Africa at IITA Nigeria in November 2018, where George participated in advising on developments of stage gates (Road), product profiles (Destination) and variety replacement strategies, as well as the RACI plan to support these. While IITA has submitted to EiB her product profiles and variety replacement strategies for cassava for food, for industry and for biofortified varieties, the other NextGen partners in the NARS have each developed a draft product profile for eventual submission by October 2019.

During our annual meeting 2019 in February in Kampala, Uganda, we had the pleasure of the company of George, Biswanath Das, Eng Hwa (Hwa had earlier participated in 2 previous annual meetings). Workshops on how to set up and organize product advancement meetings were facilitated by the EiB members and members of NextGen EPAC (Dave Meyer and Carlos Iglesia) who belong to private seed industry. Through discussions held at the meeting, we came up with some plans around creating a Global Cassava Product Profile portfolio to be housed in Cassavabase, to be delivered before November 2019.

We competed for the EiB call on Development Trait Prioritization as Part of a Sub-Saharan African Crop Variety Replacement Strategy and secured the co-funded grant for cassava in Uganda (IITA and NaCRRRI). We have made advancements in negotiating the details of the contract with AbacusBio and EiB.

In Module 3 (Genotyping / sequencing tools and services), we have worked with Eng Hwa. He has been very instrumental in negotiating and developing a working deal for the project via IITA (and RTB) on procedures for submission of leaf samples to Intertek for genotyping. Other developments include the development of a core set of QC SNPs used for fingerprinting of cassava, and the validation of markers for forward selection - virus disease, dry matter, beta carotene, etc.

In Module 4 (Phenotyping tools and services), we just started discussion with leads Gustavo Teixeira and Steve Corack. NextGen Cassava shall be on a joint mission with Module 4 on a trip to Syngenta Facility, Sao Paulo and Embrapa in Cruz das Almas, Brazil in August 2019. The purpose of the trip included:

- To observe and prioritize for adaptation certain mechanization procedures for seedling nurseries establishment and initial screenings for early generation populations.
- Robust micropropagation processes or techniques.
- The key performance indices (KPIs) for control of these procedures.

We need to further define how best NextGen and EiB would collaborate to make the best learnings for phenotyping, especially as it relates to root quality traits among others which is key to adoption of new varieties.

We have a plan to visit the Syngenta Sugarcane facility in Sao Paulo this summer for cross-learning on mechanization of seedling nurseries establishment and initial screenings for early generation populations.

Please see Appendix 1 Part 1G for more details on NextGen/EiB interactions.

RESEARCH DIVISION

The progress made on Year 1 Research Division project milestones is shown below, organized by research-focused outputs.

Breeding process map and quality management: Breeding process maps of all NextGen activities identifying responsibilities and communication requirements.

A quality champions (“Q-Champs”) team has been assembled and met regularly during Period 1. This team was responsible for adoption of barcoding. The team is also responsible for developing and reviewing of standard operating procedures (SOPs) and has made progress in developing them for the different project partners. More efforts are needed in the timely delivery of quality control documents (such as SOPs and Process Maps). We are working on ways to adjust the Q-champs team so that there is more investment from each program. Tracking and audit process maps are also in development together with integration in Cassavabase.

Genomic Predictions and Selection Checklist

In an attempt to standardize and document data management procedures used for genomic prediction and selection, Marnin developed a standard operating procedure / checklist document for use by partners. The goal was to ultimately include a code-base and store the information on Cassavabase. The document was shared in December 2018 with the Q-champs on Google Drive and Slack and should eventually lead to its routine use by partners.

Initiated “Finalizing” Phase 1 data

In late 2018 and early 2019, Marnin began working with each breeding program’s Q-champs to enact some improvements to their respective datasets, mostly from Phase 1, which were already uploaded on Cassavabase. He provided feedback based on his experience with the data, and Q-champs began filling in key historical metadata. This includes dates of planting/harvest, plot sizes and spacing (critical for correct calculation of yield) and other critical information for the future usefulness of the data. Progress has been made but a major 2019 goal is to complete the work.

Data Quality / Status “Snapshot”

For posterity’s sake, Marnin produced a “snapshot” of the NextGen Cassava data on Cassavabase as of January/February 2019. All field plot and metadata were downloaded for each breeding program (IITA, NRCRI, NaCRRI and TARI). Using the best of his knowledge and ability, Marnin summarized the amount, accuracy and quality of phenotype and meta-data that is available. The results of that work were shared as part of his presentation (Q-Champs) at the NextGen Annual meeting. Improvements in the future can be measured by re-doing the summary and comparing to previous results.

Better GS models: Time from seedling to multi-location uniform yield trials shortened by breeders through use of better prediction methods.

Breeding programs have not agreed about wanting predictions to skip an evaluation year to arrive at UYT and national variety testing trials earlier. Marnin calculated genomic estimated total genetic value (GETGVs) for the C1a of NRCRI to make selections for advancement from AYT to UYT. No evaluations yet seemed appropriate to be skipped. There has been some consultation about which evaluation stage might be the best to skip (CET, PYT, AYT), or how such a skip might impact prediction accuracy on the population improvement side of the breeding programs. We do not think that this is a good idea given that we are dealing with a clonal crop that has not reached optimal plot sizes due to the bottleneck of insufficient planting materials at the early generation breeding stages.

In Period 2, Marnin will continue to support partners in making selections across breeding stage gates for variety development and advancement. In 2019, that will entail a major new computational task, the imputation of GBS markers based on DArTSeqLD observations. In consultation with programs, genomic estimated total genetic values will be estimated for clone advancement from one stage gate to the next. At the moment, efforts are not adequately underway to fully use MAS during the coming June selection season in Nigeria except for in cases like CMD. The same scenario applies to East African partners in their October selection season. In addition to previously-used truncation selection on GEBVs for parent selection for crossing, we are interested in optimal contributions selection (OCS). OCS should enable us to increase selection intensity while maintaining diversity. Discussion needs to occur with breeding programs on the need to make specific crosses and contingencies in case those crosses fail.

Please see Appendix 1 Part 2A for more details on the Jannink Lab’s work on GS modeling.

Improved trial design: Higher accuracy data generated due to improved NextGen field trial design and layout.

Trial generation tools are available in Cassavabase; the vast majority of trials are being created directly in Cassavabase. Please continue reading below in the "Full Use of Cassavabase" milestone section to see more information on Cassavabase developments related to trial design.

PhenoApps - Cassavabase integration: Communication between Cassavabase and PhenoApps developers with breeders match tool development to breeders' needs.

Project scientists at the Makerere AIR Lab have been able to develop and test the Image Phenotyping tools in this reporting period. This year, the AIR Lab has mainly focused on prototyping, exploration and testing of the applications. As they progressed within the year, they realized that some activities depend on other teams in the NextGen group. For example, they depend on the BrAPI and FieldBook teams in order to execute their integration, and some of these dependencies along the usage of the applications can delay achievement of the set plans. Secondly, as a part of an academic institution, Makerere University, it has been necessary for the researchers to have interns working on this project as part of capacity building.

Despite these challenges, the AIR Lab has been able to start on the integration of their applications with Cassavabase and FieldBook. In the second year of the project, AIR Lab team members will spend time at BTI to work on the integration where the functionality for CBSD phenotyping will be added to Cassavabase. This will enable direct integration of our database to a format that is compatible with Cassavabase. Additional Year 2 AIR Lab activities will include:

- Based on year 1 results, selection of five trial experiments to try image-based PhenoApps data capture.
- Training and deployment of necrosis detection technology to test users at the trial sites in Uganda and Tanzania.
- Integration of PhenoApps usage tracking in apps to understand field usage.
- Further integration with Cassavabase (to enable transmission of images from the field) and Fieldbook (Fieldbook team update the FieldBook application in order to have the integration reflected).
- Evaluation of a multi-site trial of PhenoApps.

The overall goal of the AIR lab in the second year is to carry out multi-site trials of the Image Phenotyping tool and eventually have this tool out in the breeders' hands.

NIRS developments:

At IITA, handheld NIRS has been procured and is under testing for field use.

Three key phenotyping results were attained at NaCRRI: a) training workshop on use of NIRS for root trait phenotyping conducted at Namulonge; b) both bench-top and hand-held NIRs used to generate spectra data using on-station cassava field trials; and c) generated CBSD root necrosis imaging data using PhenoApps developed by Makerere University group. NRCRI team members participated in PhenoApps training workshop, and phenotyping of all field trials is now conducted using the mobile tablets.

EMBRAPA NIRS predictions: Several cassava clones were phenotyped for important agronomic traits such as carotenoid content, dry matter content, starch yield, and cooking time. NIRS spectra of these samples are being used to build prediction models using portable equipment (ASD and Scio) and benchtop (Buchi NIRFlex500). Moving forward, NIRS data will be implemented for beta-carotene, dry matter content, cooking time, and starch yield.

Together with the RTBfoods project, we will be co-hosting a two-week NIRS training workshop for users at IITA in June 2019 with resource persons from CIRAD and CIP. Trainees will be drawn from IITA, NaCRRI, NRCRI and WACCI. The purpose and scope of the training include:

- Signal enhancement and treatment
- Spectra and data base management
- Calibration development (from spectra to equation)
- Software training (at least Winisi, may be Unscrambler and/or Xlstat)
- Handle data formats according to machines
- Practical exercises (On prepared data sets and on institutes data sets, if available)
- Sample preparation and NIRS measurement:
 1. For intact roots (slices or whole roots) and smashed (ground) fresh roots
 2. For dried roots and processed products (to be define by partners)
- Characterization of homogeneity of the samples and repeatability of the spectra
- Handle acquisition and data management (from measurements to database)

- **Instrument standardization:** between portables ones, same brands (ASD) and between portables (ASD) and benchtop instruments (FOSS).

1. Theory of standardization
2. Practical exercises

Please see Appendix 1 Part 2B for more details on PhenoApps development at the AIR Lab, NIRs phenotyping for end-user traits at NaCRRRI, and NIRS predictions at EMBRAPA.

Full use of Cassavabase: digital ecosystem implemented through Cassavabase, providing design and tracking tools for all phases of the breeding scheme.

The Boyce Thompson Institute team continues their work from Phase 1 to implement a breeding database, Cassavabase (<https://cassavabase.org/>), capable of supporting the Genomic Selection use case for the project and other breeding paradigms. Cassavabase has been operational for more than 6 years and many improvements have been implemented with breeders' feedback over the years. This year also we have made a lot of progress in the reporting period in adapting new features that respond to breeders' needs, as well as supporting the breeders and users with workshops and staff exchanges. A large number of suggestions were made to the development team, and many of which will be implemented. The suggestions, bug reports, and work in progress is tracked through the GitHub issue tracker at <https://github.com/solgenomics/sgn/issues>. The areas improved include tools to search, manage and analyze phenotyping as well as genotyping data. Additional improvements were made on the backend database on how certain types of data are stored for improved query ability; these changes are less evident to the user than interface changes but are important to keep the database to perform and scale reasonably in the future.

Quality Control and data curation: a continuous effort on curating trial data is led by data managers in collaboration with PhD students and the developers to improve quality control and accuracy. An important aspect of quality control is to have a continuous information flow in Cassavabase, from field design to phenotyping to data analysis, such that all data are always in a digital format. Cassavabase recently moved its documentation to an interactive page (<https://solgenomics.github.io/sgn/>) and project document repository has been added (<ftp://ftp.cassavabase.org/documents/>).

The total data content is now over 11.6 million phenotypic observations from 3,574 trials, that evaluate 332,810 accessions on 684,500 plots. In terms of genotyping data, 32,000 accession have been genotyped. These genotyping data are being called against the new version of the genome (version 7.0) and will be updated soon. 317 genotyping plates have been added to the database, most containing 95 samples each (plus one blank well), for more than 30,000 additional genotypes. Most of these newly uploaded plates are for a low-density Intertek assay for quality control purposes.

At IITA, 172 phenotyping trial datasets and 27 genotyping trials were uploaded. breeders and field research associates use Cassavabase as the primary breeding database to manage breeding trials as well as the genotyping generated from NextGen Cassava project. Following the field plot barcoding implementation for Genomic Selection trials last year, in 2018 IITA has expanded barcoding to all its cassava breeding trials. Use of the Intercross is also being assessed (see "Crossing tools" section in the next outcome below). More than 200 tablets and phones have been deployed for field data collection and other related data management activities.

At NaCRRRI, 27 phenotyping trial datasets and 27 genotyping trials were uploaded onto Cassavabase. There has been a steady increase in the amount of trial datasets submitted onto Cassavabase through the project life (2012 to 2018). In the next period, NaCRRRI plans to submit metadata to Cassavabase; complete analysis on error rates of trials; and generate data that incorporates use of covariates.

At NRCRI, 44 phenotyping trials and 47 genotyping trials were uploaded. The NRCRI team also utilized the Cassavabase for designing all field trials and generating barcode labels. Four team members participated in Cassavabase training. The database manager (Adeyemi Olojede) undertook an extended 1-month training visit to BTI.

At CIAT, 885 phenotyping trial datasets and 30 traits were uploaded.

TARI Tanzania and EMBRAPA Brazil joined NextGen more recently. TARI has now 64 trials and EMBRAPA has 10 trials in Cassavabase. Currently, 90% of the EMBRAPA's breeding program has been using these tools for trial design, phenotyping and data collection in the field. Moving forward, all of EMBRAPA's trials will be primarily designed on Cassavabase, and 10 students/technicians have been trained on Cassavabase and PhenoApps tools in Brazil.

To enhance usage of the database, several trainings were conducted in 2018 across all locations for breeders and research technicians, including training on Fieldbook and Coordinate PhenoApps. A workshop was held at the NextGen annual meeting in Kampala in February 2019. Annual data manager training will be held at BTI in June 2019. Visits from Cassavabase developers to breeding programs is planned for September 2019.

Please see Appendix 1 Part 2C for data on Cassavabase website usage, and further details on search tool developments.

Reduced errors: Genotyping error rates reduced and trait heritabilities increased across NextGen breeding programs.

Data collection

A key process in Genomic Selection is the collection of genotyping samples and their correct assignment to accessions in the database, so that phenotypic and genotypic data can be correctly associated to each other. In Cassavabase, a Standard Operating Procedure has been established for sample collection, which is based on the Coordinate app from the PhenoApps group (<https://phenoapps.org/>). The process starts with a field that is barcoded. The genotyping team can then use the Coordinate App to scan barcode labels in the field and assign them to specific wells of a given 96 well plate. Once the plate is full, the Coordinate app file can be uploaded to Cassavabase, where it will appear as a “genotyping trial”. Each genotyping trial has a detail page where the plate layout, metadata and accessions can be viewed. The page also has links to send the data directly to certain genotyping providers, such as DartSeq and InterTek. In the future, we would like to expand the available providers by implementing a proposed BrAPI standard for this purpose. The standard also supports additional queries, such as about the state of a specific plate (whether it is in progress or finished, or problems have been detected, etc.).

Searching

The Cassavabase database contains genotypic information for 32,000 cassava germplasm, which have been scored against multiple version of the reference genome, resulting in about 80,000 genotypic profiles. This information is used in tools such as the Genomic Prediction tool (solGS), but was not searchable independently. We have implemented a genotype search that allows individual marker scores to be search in groups, which we call “markersets”. Markersets can be defined on the site in a simple interactive screen. The markersets are stored in the same way that lists are stored. In that way, they share many features with lists, such as the ability to make the data public to other users on the system. The format of the genotyping storage system was also tweaked. Now, all the fields of a VCF file are stored in the database. We also create an additional custom key that helps in faster retrieval for certain queries.

Crossing tools

Crossing and seedlot management: following the new cross search interface, the cross management was redesigned and a joined code development effort was initiated with the PhenoApps team, who have developed the Intercross app. As with FieldBook and Coordinate for phenotyping and tissue sampling, the Intercross-Cassavabase pipeline aims at integrating crossing block activities to the digital ecosystem using BrAPI. A first field test of Intercross-Cassavabase was performed at IITA Ibadan at the end of 2018, further development are implemented. The Cassavabase platform also supports btract, a system to track crossing specifically designed for banana, which consists of more complex crossing use cases than other crops.

Analysis tools

A major focus in the reporting period was on improving the analysis tools in the database. We made many improvements to the genomic selection pipeline (solGS tool), including the addition of a PCA tool for both phenotypic and genotypic data. We have also added a GWAS tool, which is based on an R package. Although the tool is usable, it turned out to be quite slow, so we are re-implementing is using non-R code that is much faster. We are also working on a mixed model tool that will allow analysis of trial data using user definable mixed models, with the ability to store the resulting BLUPs and adjusted means in the database. It will be possible to further use these results in the database using tools such as the Graphical Filtering tool or the Selection Index tool, as well as to compare the results to other data in the database using the Trial Comparison tool, etc.

Barcoding of fields

A key achievement in the reporting period is that now many breeding programs routinely barcode all their fields with the Cassavabase barcode system. This leads to much improved data quality, because data entry errors can be reduced when scanning barcodes. We established specific protocols to cheaply and durably barcode plants or plots. The barcodes are printed with standard laser printers on special plasticized labels that can be wrapped around the stem of a plant. Cassavabase provides a barcode label editor, with which customized label designs can be produced and stored/shared for future use. The barcodes have been shown to persists for many months even under high sunlight and other relatively extreme weather conditions. In the digital ecosystem, barcoding is an essential step so that all operations in the field such as phenotyping, crossing, and sample collection, can be performed to high data quality standards.



Figure 2: A cassava plant in a field in Namulonge, Uganda, tagged with a Cassavabase barcode.

SOP for trial planting verification

QC fingerprinting: IITA developed a low-density SNP panel for routine germplasm fingerprinting and quality control. These 36 markers (2 per chromosome) have been used to fingerprint more than 10,998 samples including seedlings, breeding germplasm and regular field plots.

To develop the QC/fingerprinting panel, we started with more than 70,000 GBS SNP markers screened across 1,400 cassava clones representing Africa-wide cassava breeding lines and landraces as well as Latin American germplasm. We sequentially filtered and selected a subset of SNPs that meet the following criteria:

- Minor allele frequency greater than 0.30
- Polymorphic information content greater than 0.35
- Conformance to expected genotypic frequencies based on Hardy-Weinberg Equilibrium expectation (P -value > 0.01)
- Remove linked markers (linkage disequilibrium, $R^2 < 0.2$)
- Screening for consistency of allele scores on sets of identical clones genotyped redundantly using GBS
- Uniqueness of SNP flanking sequence (BLAST against reference genome).

Using these criteria, we chose 10 markers per chromosome, making a total of 180 SNPs for the 18 chromosomes. One hundred base-pairs flanking sequences around the genomic positions of each SNP marker were submitted to Intertek for design and conversion to allele specific competitive PCR assays (KASP) assays. The performance of the designed assays was validated using DNA from 188 diverse cassava accessions. The low-density SNP panel was used to genotype our entire collection of historical breeding lines (Genetic Gain, $n = 810$), landrace collection (855), Genebank core collection (319) and advanced selection from our genomic selection pipeline (72). We obtained good quality SNP dataset with high overall data recovery rate (98.64%). The average minor allele frequency was 0.438 and proportion of heterozygotes was 0.479.

As of yet, the QC SNPs have not been used for genotype mislabeling analysis. IITA has sent QC data to the Jannink Lab and the data look promising for this purpose. A tool will be developed in the next six months (hopefully in the next two months). We proposed an SOP for trial planting verification using the QC SNPs. This will be implemented before the end of July 2019.

Please see Appendix 1 Part 2D for more details and data on genotyping developments at IITA and TARI.

Flowering: Increased flowering and seed set protocols identified and tested resulting in increased cassava crossing efficiency while maintaining optimal plant types.

At Tim Setter's lab at Cornell University, the following achievements were made:

- Lamp systems with red LEDs were installed at IITA-Ibadan and at NRCRI for extended daylength. As described in the detailed report, Setter's team used these systems to determine the extent of benefit of long-day photoperiods on the time to flowering and flower prolificacy. They also tested a range of light flux densities. These studies provided evidence of benefit in the field and are encouraging as a future tool in breeder's nurseries.
- PGR and pruning trials were led/supported by Cornell personnel and performed in three field locations IITA-Ibadan, NRCRI-Umudike, and NaCRRI-Namulonge. Support work was also performed at Cornell University on: a) the use of combined or

separate treatments of pruning, STS and BA for flower regulation; b) elucidating the mechanism of pruning benefit by testing hypotheses that it involves photosynthate status or auxin flux; c) cytokinin formulations; and d) temperature effects on flowering. These studies demonstrated benefit of all the treatments we have advanced, and show that substantial improvements in flowering are achieved when they are employed as a full package. For example, when STS, BA, and pruning were applied in IITA-Ibadan field plots, the number of flowers in the first tier was increased from less than three in the controls to about 30 in the full treatment. See detailed report.

- Protocols for pruning and PGRs were written and distributed for review.

In the next reporting period:

- At Ithaca, greenhouse trials will be conducted to determine a) appropriate dosage and timing of BA in conjunction with pruning to limit hermaphrodite formation; b) interaction of plant photosynthate status with PGR and pruning; and c) treatments that will improve fruit and seed set.
- At Ithaca, growth chamber studies will determine effects of photosynthate status, as manipulated by root zone temperature, atmospheric [CO₂], and light flux density, on flowering;
- Ithaca-based crew will collaborate with IITA, NRCRI, and NaCRRRI on field trials. These will involve: a) design, establishment and testing of red LED systems; b) pruning + PGR best practices;
- Ithaca-based crew will provide guidance, advice, and coordination of flower/seed work at Africa and other stations. This will involve travel to some of the sites, Zoom calls, and email.

In July 2018 at CIAT, the infrastructure for a large red light treatment (RLT) nursery was completed. A total of 24 red LED-light reflectors (50w) were installed to illuminate an area of 1863 m². A set of 190 clones were planted under this RLT for crossing activities. Of those, 150 clones were also planted nearby, under normal photoperiod conditions. The purpose of this setup was to assess and compare flowering patterns with or without extended photoperiod in a large sample of genotypes. Erect clones (e.g. those that fail to flower or flower very late under normal photoperiod conditions) responded positively to RLT (e.g. flowered earlier and thus height of first branching was lower). On the other hand, bushy types (e.g. genotypes that flower early and profusely) tended to be indifferent to RLT or react negatively (e.g. flower later under RLT).

Data on the impact of RLT on four genotypes was generated at CIAT for the third consecutive year. An additional experiment compared the effect of RLT alone, or in combination with pruning of young branches in the first or second branching events, with or without the addition of BA the day of pruning. RLT during the night resulted (as already demonstrated during the past two years) in earlier branching of otherwise erect genotypes. This earlier flowering allowed pruning young branches much earlier than plants growing under normal photoperiod. Fruit and seed set can be obtained from the first branching event, which is generally sterile if branches are not pruned. The highest number of seeds produced was always in a treatment that included extended photoperiod, pruning (either in the first or second branching) and with the application of BA on the pruning day. There is an interesting pattern in the response to pruning. In the intermediate flowering genotype, clearly the best results were obtained by pruning at the second branching event. In the late flowering clone, pruning in the second branching event yielded higher number of seeds but not considerably higher than pruning at the first. In the case of the very late flowering genotype, on the other hand, pruning at the first branching event was clearly the best option. With the information generated in 2019, CIAT will prepare during 2019 (and before) the following manuscripts:

- Effect of red light treatment and night breaks (based on three consecutive years of data)
- Effect of pruning young branches in combination (or not) with red light treatment. Combining data of two years (at least for one genotype).

A second field evaluation of flowering and fruiting in the EMBRAPA's genebank was begun in 2018. These evaluations aim to correlate flowering phenology with climatic factors to characterize the germplasm suitable for crossing and genomic studies. Five contrasting varieties for flowering were planted in the field, where they will be submitted to different treatments to increase flowering rate and seed production, such as extension of the photoperiod with red light, premature pruning of the branches, and application of BA and STS. Installation of field facilities with red light reflectors is planned for the next period, to carry out photoperiod extension studies in combination with pruning and application of growth regulators.

Flowering experiments were undertaken by African partners as well. In evaluating flowering manipulation methods for potential use in practical breeding, NRCRI conducted 3 different studies which evaluated effects of different treatments on flowering and seed set. These include trials on the effects of red light treatments, the role of PGRs application method and the effect of pruning. In the next period, evaluation of the best treatment combinations for the effect of red light and PGRs on flowering and seed set will be made using large field trials. At NaCRRRI, a set of 20 varieties of varying flowering habits were selected and planted to evaluate the effect of plant growth regulators (PGRs) and pruning on enhancement of flowering and seed set. NaCRRRI plans to continue testing the hormones STS + BA in the crossing nursery, and implement the lamp system to enhance flowering and seed set.

Please see Appendix 1 Part 2E for more details and data on flowering experiments at the Setter Lab, CIAT, EMBRAPA, NRCRI, and NaCRRRI.

Pipeline optimization: Standard operating procedures for breeding pipeline developed.

At the 2019 Annual meeting, each breeding program nominated contacts to begin work. For IITA, Moshood Bakare has estimated error variances from different trial types. Prompted by a query from Gary Atlin in July 2018, IITA, NRCRI, NaCRRRI, and EMBRAPA provided cost parameter estimates. These were explicitly back-of-the-envelope. CET plot cost estimates ranged from \$8 to \$10. UYT plot cost estimates ranged from \$24 to \$73. Somewhat more rigorous approaches to estimating plot costs were discussed at the 2019 Annual meeting. More communication within the groups should continue before reaching mutual agreements on acceptable cost estimates. GxE analyses were performed by Uche Okeke on IITA data (Mokwa, Ibadan, Ubiaja) in 2016 / 2017. Such analyses are ongoing for NaCRRRI by Alfred Ozimati. We have not developed a standard analysis that could be applied uniformly across programs and this is a target for period 2.

CIAT's commitment was to share experiences with the F1C1 approach and its potential for NextGen. The advantage of this innovation has been shared with NextGen partners in every possible forum. Preparation of a Manual on Quantitative Genetics is ongoing. Six chapters are already available and should be ready by end of Period 2.

GXE work at NaCRRRI: a) GxE estimates generated for AYT trials of pVAC and white-fleshed cassava clones, and b) error variances estimated for selected traits being evaluated in different stage-gate trials are due for completion by end of Period 2.

Please see Appendix 1 Part 2F for more on CIAT's approaches to increase heritability.

Prediction methods and mating designs: Prediction methods and mating designs developed and tested to account for cassava gene action and geographic diversity.

Non-additive model predictions were made for NRCRI in October 2018. Moshood Bakare (PhD student with Jean-Luc Jannink at Cornell) is conversant now with these models and should be able to perform them for IITA in Period 2. Other breeding programs have not yet completed their analyses using these models otherwise. Marnin has initiated research on using deleterious allele models for predictions. These results should be available by the end of the next reporting period. The Buckler Lab has hired a new postdoc, Nisha Singh, who will also be working in that space, in addition to working on a cassava PHG.

Genotyping: Standard operating procedures for genotyping developed to generate quality genotypes and best predictions

At the Buckler Lab at Cornell University, whole RNA-seq across 5 developmental stages of 6 cassava clones were successfully collected and sequenced for transcriptome and to annotate genome assemblies. The 3' mRNA from 150 diverse set of training populations from selection 5 developmental stages were sequenced successfully and send the analysed data to NaCRRRI for TWAS analysis.

Transcriptome wide association analysis (TWAS) and genome annotation

For transcriptome analysis, genome assemblies and annotations of cassava against v.7 performed whole RNA-sequencing and data provided to JGI. The paired end 150bp RNA-sequencing was done for six cassava clones from NaCRRRI (EBWANATEREKA, Nase14, TME204, TME419, Nase3 and TMS1980002) from five different tissues including leaf, stem, meristem, fiber root and tuber.

3'-RNAseq data was generated from total 150 cassava clones in five tissues for each clone (150x5 = 750 tissues) including leaf, stem, fibrous root, tuber and flower. These genotypes are from NaCRRRI few individuals are the part of training population of breeding program. Expression data of all the genes across all individuals were generated for transcriptome wide association analysis (TWAS) in different traits data send to NaCRRRI.

Genotyping: Suitable bioinformatics tools and pipelines developed for calling alleles, rare allele imputation, and genetic load identification.

This work is led by the Buckler Lab at Cornell University. A shift in mid-density genotyping platforms changed the focus for the year to ensuring that a DArTseqLD could be used as the primary genotyping platform for GS. High imputation accuracy between platforms was achieved. Substantial progress was made on PHG development, and strategies for phasing haplotypes for the PHG were tested during the year. A HapMap 3 using the latest genome version is in progress. The Buckler Lab has identified ~5000 loci in Cassava genome with high PIC to develop mid-density genotyping platform including 140 trait specific SNPs from NextGen Phase 1, including all forward breeding markers.

The Buckler Lab has completed all planned tissue collection and sequencing for expression profiling using RNA, including whole RNA sequencing from developmental stages and 3' RNA from 150 lines. The practical haplotype graph has been implemented in other species including maize and sorghum. A practical haplotype graph has been created in cassava and is undergoing testing and refinement. Significant portions of the cassava genome are found in identical by state stretches that we plan on using to extract haplotypes for the practical haplotype graph. We are currently creating a HapMap3 which will include whole genome sequencing from over ~600 cassava lines at varied depths which will contribute to multiple projects including the practical haplotype graph, association

mapping, and deleterious allele discovery. We have planned to collect tissue and sequence multiple species within the Euphorbiaceae family for comparative genome analysis and genome evolution across 18 Euphorbiaceous plant family. With the implementation of a new marker system, imputation between the new markers (DarTseqLD) and old markers (GBS) was necessary. We tested the imputation accuracy between marker sets and refined methods to obtain high accuracy. This also allowed us to verify the agreement between genotyped taxa identification.

Imputation

For a number of reasons, mid-density genotyping GBS had to be shifted to DarTseqLD. Dr. Wolfe focused on imputation accuracy on genome wide prediction. Our group focused on imputation accuracy at the SNP level. In year one, we focused on the imputation between separate marker sets – GBS, DarTseqLD, and HapMap 2 (WGS). To test the effectiveness of imputation between marker sets we used data from the EMBRAPA program that had been genotyped by both technologies. By developing and implementing filters based on site missing data, Hardy-Weinberg, identifying high error individuals and sample mix-ups, overall accuracy was improved from <80% to over 95%. Imputation accuracy is expected to further increase based on the relatedness between the training populations and the imputed taxa. We are currently genotyping many of the parents in the breeding parents to improve imputation between WGS, GBS and DarTseqLD.

Practical Haplotype Graph

The key value proposition of the Practical Haplotype Graph (PHG) is to allow consistent imputation of common and rare alleles without vendor lock-in of genotyping platforms, and for potentially enabling very low-density genotyping to be effective in genomic selection. The development of breeding PHG has been completed for maize and sorghum. Progress has been made in the process of extracting and collapsing haplotypes for pathing. Test PHG databases have been created for cassava, but are undergoing continued development and refining to overcome the initial phasing haplotypes of heterozygous and clonal cassava. Current efforts are focused on improving phasing with several approaches: 1) Despite cassava being a highly heterozygous species, breeding approaches have resulted ~15% of each genome to be homozygous state. Combined with the HapHap below this permit the phasing ~100 haplotypes at each gene. 2) Phasing haplotypes will also be identified through 2-3 generation pedigree relationships (see HapMap below). 3) Low depth long read sequencing might prove viable as a method to phase highly heterozygous genomic regions and for parents without extensive pedigrees.

Third-generation cassava haplotype map (HapMap3)

We are creating a comprehensive third-generation cassava haplotype map (HapMap 3) which comprises ~679 diverse cassava accessions and based on the newest assembly (v.7). To maximize the diversity and representation for cassava, all samples were selected on the basis of breeder's choice and diversity analysis from accessions included in Next Generation Cassava Breeding project of them 241 from HapMap 2 (Ramu et al., 2017) and newly sequenced 438 accessions including Trios-90 and NextGen parents 348. In this context, we have piloted the GATK 4 Sentieon pipeline for cassava HapMap 3 with few accessions. It will be a valuable resource for cassava genetic studies including association mapping of disease resistance, phasing, imputation, and estimating the genetic load.

SURVEY DIVISION

Survey (phenotyping quality traits): Validated trait packages and economic weights for garri/fufu and boiled cassava informed by RTBFOODS and validated with 1000 minds.

In Phase 2 of NextGen, Survey Division adopted two new tools that had been untested in cassava: TRICOT and 1000 minds. In Year 1, the majority of our work focused on laying the foundations for these two methods, and piloting our implementation plan.

TRICOT

In Nigeria, IITA and NRCRI have worked seamlessly together to complete remaining mother baby trials from Phase 1, as well as pilot TRICOT and prepare for 1000 minds application all at once. The 2 mother and 40 baby trials in Osun and Imo states have all been harvested and processed into garri and eba, in depth evaluation of processing steps and pairwise ranking on products has been completed (Figure 3). NextGen varieties performed very well across categories. Interesting findings include precise descriptors for quality (cohesiveness, color), as well as recognizable "NextGen brand" traits such as umbrella-shaped architecture, high harvest index and thin stem. Gender and diversity focused research in Nigeria has revealed differences in roles, but also imagined futures of diverse participants (including vulnerable migrants) for cassava production.

	Osun				Imo	
	Overall	Baby trials	Mother trials	Baby trials	Mother trials	
Yield	TMS-IBA980505	TMS-IBA980505	TMS-IBA980505	TMS13F1365P0002	TMS-IBA980581	
	TMS13F1365P0002	TMEB1	TMS-IBA980581	TMS-IBA980505		
	TMS-IBA010040	TMS13F1365P0002	TMS-IBA961632	TMEB7		
	TMS-IBA961632	TMS-IBA010040	TMEB419	TMS-IBA961632		
	TMEB1	TMS-IBA980581	K195	K195		
Density/DM	TMS13F1160P0004	TMEB419	TMEB419	TMS13F1160P0004	TMEB419	
	TMEB419	TMS13F1176P0002	TMS13F1160P0004	TMS13F1176P0002	TMS13F1176P0002	
	TMS13F1176P0002		TMS13F1176P0002	TMEB419	TMS13F1160P0004	
	TMS-IBA961632		TMS30572	TMEB1	TMS13F1365P0002	
			TMS13F1365P0002	TMEB2	TMS-IBA980505	
Fresh roots evaluation (rank)		TMS13F1365P0002	TMS13F1160P0004	TMS13F1176P0002	TMS13F1176P0002	
		TMS-IBA980581	TMS13F1365P0002		TMEB2	
		TMEB419	TMS-IBA961632		Agric	
		TMS-IBA961632	K195		TMEB419	
		TMEB7	TMS30572			
Gari evaluation (rank)		TMS13F1160P0004	TMEB2	No sign difference	TMS13F1365P0002	
			TMS-IBA961632		Agric	
			TMEB693		TMEB7	
			TMS-IBA980581		TMS-IBA010040	
			TMEB419		TMS-IBA980581	
Eba evaluation (rank)		TMS13F1160P0004	TMS-IBA961632	no assesment	Agric	
		TMS30572	TMS13F1160P0004		TMEB1	
		TMEB1	TMEB7		TMS-IBA961632	
		TMEB693	TMS-IBA980581		TMEB2	
		TMS-IBA961632	LC(AtuHonorable 2)			

Figure 3. Overview of the varieties that performed well at each processing stage of the Mother baby trials. Varieties within each box are ranked from highest average (on top) to lowest average (last in the list).

The TRICOT pilot has served an important role to develop manuals and data collection plans and formats, as well as testing data collection methods. We used a stepwise purposive sampling to select bundle recipients (Figure 4). A scoping tool was used at community level will help select the most representative pool of recipients, that cover sex, age, marital status, wealth, education, ethnicity etc diversity within that community. All communities have been chosen based on interaction with ADP, all communities have been visited and lead farmers have been selected with purpose sampling of the different social groups. A lead farmer guide for the agronomic evaluation for 1, 3, 6 and 9 months after planting has been prepared (Figure 5). To select TRICOT varieties for Nigeria, BLUPS and BLUES were generated for 14 different traits, along with quality data from UYT trials and lastly where possible on farm quality evaluation were triangulated to select the final lines for distribution.

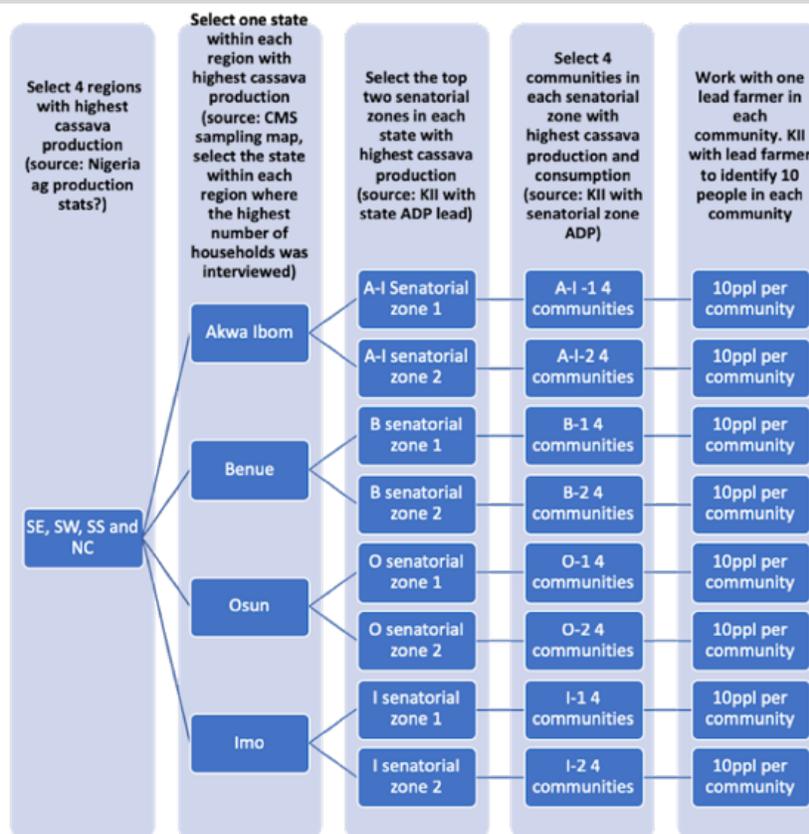


Figure 4. Sampling frame for Nigeria TRICOT. A similar approach was followed in Uganda.

Time after planting	Trait/characteristic
1 month	<p><i>Overall impression</i></p> <ul style="list-style-type: none"> • Germination/Sprouting • Plant growth • Weed competitiveness/canopy formation • Suitability to soil and environment
3 months	<p><i>Overall impression</i></p> <ul style="list-style-type: none"> • Survival/sprouting • Plant growth • Weed competitiveness/canopy formation • Branching habit/architecture of the plant • Disease resistance (deformation of leaves etc)
6 months	<p><i>Overall impression</i></p> <ul style="list-style-type: none"> • Survival/sprouting/nr of plants left • Plant growth • Weed competitiveness/ canopy formation • Branching habit/architecture of the plant • Disease resistance (deformation of leaves etc)
9 months	<p><i>Overall impression</i></p> <ul style="list-style-type: none"> • Plant growth • Weed competitiveness/ canopy formation • Branching habit/architecture of the plant • Suitability to soil and environment

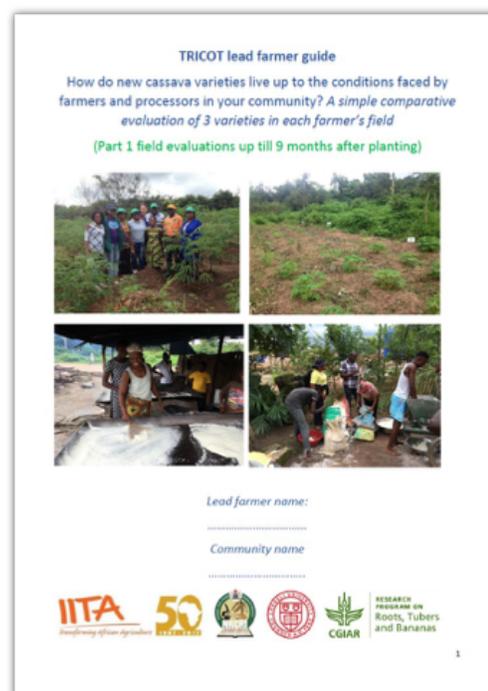


Figure 5. Example list of traits for evaluation 1-9 months after planting (left) and TRICOT evaluation manual (right)

In Uganda a total of 240 diverse cassava farmers selected from Central, Northern and Eastern regions have enrolled for trials. The farmers were selected through an identical step wise purposeful sampling frame presented above for Nigeria: Each region 2 major cassava growing districts leading to 6 districts in total. KIIs conducted with district agricultural officers (DAOs) were purposively select sub-county/ district. Guided visits made to farmer groups that were selected by sun country AOs. All the members in the farmer groups

were interviewed and selection made with 10 participants select per parish based on sex, marital status, ethnicity, willingness to participate and amount of land allocated to cassava farming. Collectively, farmers are evaluating 12 cassava clones (9 from NARO and 3 from IITA), as well as replacement and local checks. Packages were developed in CLIMMOB (Figure 6).



Package code	Variety A	Variety B	Variety C
Package #1	UG120198	UG120193	NAROCASS 1
Package #2	UG120024	UG130016	MM06/123
Package #3	UG120156	UG120180	UG130007
Package #4	UG120124	MM16/0707	MM16/1627
Package #5	UG120180	UG120198	MM16/0707
Package #6	NAROCASS 1	MM16/1627	UG120124
Package #7	UG130007	MM06/123	UG120156
Package #8	UG120193	UG120024	UG130016
Package #9	NAROCASS 1	UG130007	UG120180
Package #10	MM16/0707	UG120156	UG120124
Package #11	MM16/1627	MM06/123	UG120198
Package #12	UG130016	UG120124	UG120193
Package #13	UG120156	UG120193	UG120024
Package #14	MM06/123	UG130007	MM16/0707
Package #15	UG120198	UG120180	MM16/1627
Package #16	UG120024	NAROCASS 1	UG130016
Package #17	MM16/0707	UG120198	MM06/123
Package #18	UG120124	UG130016	UG120193
Package #19	MM16/1627	UG120156	NAROCASS 1
Package #20	UG120180	UG120024	UG130007
Package #21	UG130016	UG120198	UG120180
Package #22	UG130007	MM16/1627	UG120156
Package #23	MM06/123	UG120124	UG120024
Package #24	UG120193	NAROCASS 1	MM16/0707

Figure 6. Example of Package list for Uganda TRICOT (first 24 packages listed).

In Tanzania, TRICOT is being piloted in two districts of coastal lowland Tanzania where twelve varieties are being tested with a total of 32 farmers in Bagamoyo and Kibaha districts. Material has been planted for the main TRICOT trial expected to begin in 2020.

Links with RTBfoods have been good, the NextGen teams in **Nigeria and Uganda** supported data collection and analysis (both continuing) for WP1 and WP5 activities. Demands on the NextGen team’s time, as well as demands on the NextGen budget to support RTBfoods activities has been a challenge. We are working to balance deliverables under NextGen, and those under the RTBfoods mandate. For both Uganda and Tanzania, traits to be evaluated by TRICOT post harvesting (harvesting, processing, consumption) will be informed by outputs from RTBfoods WP1 Activity 3 final reports that are currently in development. We anticipate by this time next year these traits will be well defined and validated for use in TRICOT. **In Tanzania**, the TARI team has implemented the RTBfoods WP1 tools (SoK, gendered food mapping and market interviews) the report is expected imminently.

Plans for next reporting period:

In Nigeria: The 300 TRICOT trials will be established in 2019, preparation for the 1000 mind study has been initiated through 16 focus group discussions in the 8 largest cassava producing states in Nigeria. We anticipate to publish up to four publications, at various stages of preparation and submission within the next reporting period. Fieldwork for MSc student Duro Owoade will be completed in the next reporting period. Co-funding through RTB CRP will help support complementary gender research and TRICOT scaling activities. **In Uganda**, data will be collected from TRICOT trials and 1000minds survey will be implemented on all recruited farmers, with an additional ~200 others (with co-funding from EiB). **In Tanzania**, the RTBfoods WP1 survey work will be completed, and the TRICOT pilot will be monitored and method adjusted for scaling in 2020.

Please see Appendix 1 Part 3A for more data on survey research in Nigeria, Uganda and Tanzania, including TRICOT evaluations.

Gender (research capacity development): Gender responsive research capacity building workshops and comparative gender research in Nigeria, Uganda and Tanzania.

This milestone has been delayed. As mentioned above, in Year 1 the survey team focused heavily on establishing TRICOT as a viable on farm testing method for cassava, concurrent to finishing Phase I remaining gender research, and supporting RTBFoods activities. Together these activities saturated the team members, thus we have delayed the gender training plans for next reporting period. We are

currently working with Makerere university to plan a GREAT-like training in Nigeria for IITA and NRCRI in 2019. We plan trainings in Uganda and Tanzania in 2020.

PROJECT MANAGEMENT

Project management, internal/external communication: Increased communication bandwidth and accountability within and across NextGen partners.

As in previous years, we held a week-long annual meeting. This year's, held in Kampala, Uganda with the NaCRRI team as our hosts, we had over 100 project partners and collaborators in attendance. This meeting is our main event of the year, and allows almost all project participants to be physically together as we review the year's achievements and challenges, and plan for the next year's activities. We had presentations representing all partner groups, and involvement from collaborator projects/groups such as EiB, the African Cassava Whitefly Project, RTBfoods, CASS, and CIP, as well as five CoPP members. We had five extended work-planning sessions, and a half-day EPAC challenges session. We ended the meeting with two days of workshops (including Product Advancement, Cassavabase, PhenoApps, TRICOT, and CoPP work planning) and an RTBfoods side meeting. Our field trip to Namulonge allowed all partners to see the facilities at NaCRRI and visit cassava fields.

Throughout this reporting period, we have held regular Leaders' conference calls every other week. These calls were initiated in April 2018. Feedback from partners is that these meetings are very useful. Each call includes presentations from two project groups, who share slides which are then posted to the Leaders' Slack channel. This gives us a running repository of project activity overviews, and those who cannot make the call can reference them easily. Eder de Oliveira from EMBRAPA says, "The monitoring and constant exchange of information about the results of the project, either online or at annual meetings, has allowed a standardization of knowledge about the research advances for immediate application in different breeding programs."

We have now been using Slack as an internal communications platform for over a year. Usage continues to increase, as does the number of members. At the time of reporting, we have 110 members, with 48 of them active weekly. Certain Slack channels, such as "genotyping_breeding" have been very active, as well as the project management and leaders' channels.

The project management team, comprised of Chiedozi Egesi, Hale Tufan, and Jean-Luc Jannink (leads of the Breeding, Survey and Research divisions respectively) and Canaan Boyer (project coordinator) has been meeting monthly and has been able to monitor project progress, push for particular initiatives when needed, and plan/facilitate the annual meeting in February 2019, saving the project money spent previously on an outside meeting facilitator.

The NextGen External Project Advisory Committee (EPAC) continues to meet quarterly, and its members provide valuable guidance and stewardship. The project management team endeavors to assure that the recommendations of the EPAC are put into practice.

Web presence: Revision/redevelopment and linkage of the NextGen websites (Nextgencassava.org and Cassavabase.org); increased visibility of both sites.

After the 2018 annual meeting, the team decided there was a need to revise the NextGen Cassava website, but it was unclear to what extent it would be changed. In order to identify where improvements could be made to reflect the changes in Phase 2 and to guide the communications team in developing the website, we conducted an internal survey and held multiple meetings to discuss website revision and development. Members of the communications and administrative teams then went into an iterative process of website design according to the technical and content revisions we identified from these discussions.

Technical revisions identified for the website included a complete overhaul of the site structure, a more dynamic website interface, and transfer to a new site hosting structure (from HTML to WordPress) to facilitate site management. Content revisions identified included updating text and images to reflect Phase 2 activities, simplifying content to be more public-facing, and ensuring that project partners, donors, Cassavabase, and complementary activities were more visible in site navigation and footers. The website was officially launched during the 2019 annual meeting and has thus far received positive feedback from the overall NextGen team. We are establishing a baseline of website use through Google Analytics, and can track the site's data through the next period.

Multimedia: Stakeholders have access to regularly produced multimedia, including taped interviews, lectures, training videos, newsletters, press releases and social media posts.

The quarterly newsletter has been steadily released through this period. This newsletter offers a venue for internal project communication, as a place to widely share successes, events, and activities of interest. While the first year of issues' audience was mainly project members, we are actively seeking a wider audience, and have added 61 new subscribers in this period (from 107 to 168).

In this period, the Communications team produced 11 short videos on topics including farmers experiences with cassava diseases, searching for new sources of resistance, genomic selection, and participatory variety selection. Segments were wrapped into 15-minute documentary about NextGen in Tanzania. Work on a Uganda-focused documentary is underway.

Other multimedia produced this year includes several print media releases, such as a press release at the time of the annual meeting, and several Cornell Chronicle articles. In print media, a NextGen-focused article was released in Nature magazine.

Interviews with project scientists were aired on local news channels (and some are available on YouTube), including interviews with Hale Tufan and Jean-Luc Jannink in Nairobi at a gender and breeding conference, and with Chiedozi Egesi in Cotonou, Benin at the time of the GCP21 conference and at Namulonge, Uganda during the annual meeting.

In September 2018, we moved NextGen-related YouTube content from the general Cornell IP-CALS account to a designated NextGen Cassava channel. We've been collecting stats since the account was activated:

Youtube: <https://www.youtube.com/channel/UCqIgtCl88yvjvAxmYB7E3OQ>

Watch time 1.9k minutes

Video Views: 521

Subscribers: 23

Facebook: Page gained 376 "likes" during reporting period

Facebook Videos:

1.7k minutes watched. (up 5% since previous period)

3.4k 3-second video views (up 40% since previous period)

Additionally, CIAT has been working on producing over 90 minutes of digital training materials related to cassava breeding and phenotyping protocols. Cornell staff will work with their team to make the videos available through NextGen's channels.

CAPACITY BUILDING

Workshops: NextGen findings and accomplishments presented at relevant workshops.

NextGen scientists participate in many conferences and meetings each year, such as GCP21, PAG, the RTBfoods annual meeting, the Food and Nutrition Security Conference at University of Ghana, Excellence in Breeding meeting, ISTRC, and more. Below is a detailed table with all presentations made in the reporting period by project members, beginning with the most recent. Please see Appendix 1 Part 4A for a list of poster presentations.

Title	Author(s)/Presenter(s)	Conference/Meeting	Format
Gender Responsive Breeding and Product Profiles	Hale Tufan	Seeds of Change Conference, Canberra, Australia; 2-4 April, 2019	Talk
Executive Summary of NextGen Cassava Project	Chiedozi Egesi	RTBfoods Annual Project Meeting, Abuja, Nigeria; 21-27 March, 2019	Plenary
Qualitative Gender Research Methods	Hale Tufan	RTBfoods Annual Project Meeting, Abuja, Nigeria; 21-27 March, 2019	Training offered
Milestones Attained Towards Optimizing Cassava Breeding Programme of Uganda	Robert Kawuki	PAG XXVII, San Diego, USA; January 12-16, 2019	Talk
Establishing the foundation for molecular breeding in cassava: from discovery, validation to deployment of trait-linked markers	Ismail Rabbi/Chiedozi Egesi	PAG XXVII, San Diego, USA; January 12-16, 2019	Talk
Modernizing Cassava Breeding in Africa: the case of NextGen Cassava	Chiedozi Egesi	18th Triennial Symposium of International Society for Tropical Root Crops, Cali, Colombia; 22-25 October 2018	Talk
Genomic prediction of breeding values in R	Marnin Wolfe	GOBII meeting, Boyce Thompson Institute, Ithaca, NY, USA. 11 October 2018	Workshop
Modernizing Cassava Breeding in Africa: the case of NextGen Cassava	Chiedozi Egesi	International Conference on Food and Nutrition Security, West Africa Centre for Crop Improvement, University of Ghana; October 3-4,	Talk

		2018	
Gender Responsive Breeding	Peter Kulakow	International Conference on Food and Nutrition Security, West Africa Centre for Crop Improvement, University of Ghana; October 3-4, 2018	Talk
Genomic selection in theory and practice.	Jean-Luc Jannink, Marnin Wolfe	Excellence in Breeding (EiB) project meeting, Amsterdam, Netherlands. September 24-27, 2018	Workshop
Reducing Post-harvest Physiological Deterioration in Cassava Roots: The Nigerian Experience	Damian Njoku	FAO/IAEA Vienna, Austria; 27-31 August, 2018	Talk
Hybridization of Latin American and African Cassava Genotypes under Virus Free Conditions in Hawaii.	Kulakow, P.A., J. Norton, S. A. Motomura-Wages, T. Matsumoto, C. Hershey, C. Egesi, and H. Ceballos	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Progress and challenges in our understanding of cassava breeding and genetics.	H. Ceballos	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Plenary
NextGen Cassava Breeding: Delivering Genetic Gains in Cassava to Smallholder Farmers in Africa.	Chiedozie Egesi	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Plenary
The Burden of Trait Introgression in Cassava - Waxy Starch Trait as an Example	H. Ceballos	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Artificial Intelligence and data science - opportunities for cassava disease diagnosis and surveillance	Ernest Mwebaze	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Phenological diversity of flowering and fruiting in the cassava Brazilian germplasm	Alfredo Alves	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Genome-wide association study of resistance to cassava green mite pest and related traits in cassava (<i>Manihot esculenta</i> Crantz)	Lydia Ezenwaka	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Development of tools for cassava flowering induction and promotion	Simon Peter Abah	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Genetic improvement of cassava for post-harvest physiological deterioration	Damian Njoku	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Cassavabase - a global breeding database for Cassava NextGen breeding	Lukas Mueller	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Semi-Autotrophic Hydroponics (SAH), A New Technology for Rapid Multiplication of Cassava	Mercy Diebiru-Ojo	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Rapid Multiplication of Hardened Off Tissue Culture Plantlets For Improved Cassava Pre-Basic Seed Delivery System In Tanzania	Kiddo Mtunda	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk
Gender related attributes of cassava flour among smallholder farmers in Uganda: a case of Zombo district	Ann Ritah Nanyonjo	GCP21 IVth International Cassava Conference. Cotonou, Benin; 10-16 June, 2018	Talk

Internet bandwidth: Incremental improvements made to institutes' internet bandwidth and related technology systems lead to a strengthened and more reliable infrastructure that enables team communication and research activities.

IT specialist Stefan Einarson has visited Nigeria, Uganda and Tanzania in the last period to help build internet infrastructure and bandwidth. Based on reports from Citinet for Nigeria for the last 30 days, they have been down less than 1% of the time, we have 12 mbps going to the institution, and scientists getting 384kbps on average.

For Mwanza in Tanzania, we have 4 mbps and on average over 95% uptime. For Namulonge in Uganda, we have 15 mbps and near 95% uptime. In both of these countries, Einarson is helping them switch providers currently to improve service.

Einarson will continue to visit these institutions regularly to troubleshoot and find the best internet providers.

Capacity development (training workshops): Trained and motivated breeding personnel.

Between September 21 and October 2, 2018, Marcela Pineda (CIAT) and Peter Hyde (Cornell University) visited Nigeria (NRCRI and IITA), Uganda (NaCRRI) and Tanzania (TARI). The main purpose of these visits was to interact with colleagues from the host institutions on the different techniques to induce flowering and early fruit set. Training was based on PowerPoint presentations and, more importantly, field demonstrations.

Cassavabase trainings were conducted in 2018 across all locations for breeders and research technicians, including training on Fieldbook and Coordinate PhenoApps. A workshop was held at the NextGen annual meeting in Kampala in February 2019. Annual data manager training will be held at BTI in June 2019. Visits from Cassavabase developers to breeding programs is planned for September 2019.

Many NextGen team members participated in a hands-on training in basic quantitative genetics concepts and their implication for cassava breeding. The training course on statistics and quantitative genetics with special emphasis on RTBs took place in Uganda and Nigeria in fall 2018. The training module covered diverse type of analysis based on the use of Excel. The main advantage of Excel is that it allows trainees to understand how data is consolidated, even for the analysis of complex data sets. A key feature of RTB crops is that research focus on individual genotypes within families and data are often taken on individual plants. Specific examples were given on how to address these special features of RTBs. Scientists from different institutions in Uganda (NaCRRI, Makerere University, MaRCCI, NaIRRI, NARL, CIP), Nigeria (IITA, NRCRI), Ghana (CIP, CSIR/SARI) and Perú (CIP) representing cassava, sweetpotato, banana/plantain, and yams attended these workshops. At least 82 people attended the training in Uganda and 18 colleagues participated in the experience at IITA.

The following is a summary of other trainings and exchanges that took place in this reporting period:

Date	Visitor/Affiliation	Destination/Host	Purpose of Travel
16-29 April, 2018	Guillaume Bauchet, BTI	EMBRAPA, Brazil	Cassavabase/PhenoApps training
16-28 July, 2018	Chiedozie Egesi, IITA	NaCRRI, Uganda TARI, Tanzania	Project updates and monitoring
July 2018	Luciano Braatz de Andrade, EMBRAPA; Prasad Peteti and Afolabi Agbona, IITA	BTI and Jannink Lab, Cornell University, USA	Cassavabase training and collaboration
August 2018	Kasele Feruzi, TARI	Boyce Thompson Institute, USA	Cassavabase training
27-29 September, 2018	Peter Hyde, Cornell University (Setter Lab) & Marcela Pineda, CIAT	NaCRRI, Uganda	Flowering workshop
October 2018	Peter Hyde, Cornell University (Setter Lab) & Marcela Pineda, CIAT	TARI, Tanzania	Flowering workshop
October 2018	Marnin Wolfe, Cornell University (Jannink Lab)	Berlin, Germany	HFP leadership training
October 2018	Chiedozie Egesi, Ismail Rabbi (IITA), Damian Njoku (NRCRI)	Berlin, Germany	2018 Grand Challenges Annual Meeting

12-29 November, 2018	Adeyemi Olojede, NRCRI	Boyce Thompson Institute, USA	Cassavabase training
18-23 November, 2018	Isaak Teclé, BTI	NaCRRI, Uganda	R-training
12-16 November, 2018	Isaak Teclé, BTI	TARI, Tanzania	R-training
5-9 November, 2018	Isaak Teclé, BTI	IITA, Nigeria	R-training
27 November - 20 December, 2018	Hernán Ceballos, CIAT	NaCRRI, Uganda	Quantitative Genetics workshop
8-24 February, 2019	Jenna Hershberger, Cornell University (Gore Lab)	NaCRRI, Uganda	PhenoApps workshop

Capacity development (graduate students): African breeding programs strengthened through training of graduate students in plant breeding, sponsored by NextGen.

NextGen is currently supporting 14 graduate students in plant breeding (7 female, 7 male). Three are MSc students at Makerere University Regional Centre for Crop Improvement (MaRCCI), and two are enrolled in the PhD program there; three are at the University of Ghana's West African Centre for Crop Improvement (WACCI); three are enrolled in the PhD program at Cornell University; one is at the University of Otago in New Zealand, and one is at the University of Ibadan in Nigeria.

On July 17, 2019, MaRCCI will become the first SSA host (outside of South Africa) of the global Corteva Plant Science Seminar. NextGen students will attend this world-class seminar, and are represented on the organizing committee. The seminar is student planned and run. The broader MaRCCI student group (currently 26 MScs and 15 PhDs) has selected Chiedozi Egesi as one of the plenary speakers.

MaRCCI students will be encouraged to participate in the African Plant Breeders' Association (APBA). It is hoped that some of the MaRCCI NextGen students will find funding to attend the inaugural APBA meeting in Ghana from 23-25th October 2019. Please see Appendix 1 Part 4B for more on MaRCCI's program.

Survey division research students: MSc Student Duro Owoade has been developing a proposal to look at which varieties are moved around by local informal seed dealer initiatives. Most farmers do not buy varieties as they replant what they have been using for long, but small initiatives of stem dealing have been observed and it would be crucial to know what kind of material is traded here: what characteristics do these varieties have that make them worth moving around from place to place. Linkages with findings from the BASICS project will be made here. Furthermore, the MSc student will follow up to what extent varieties of the Mother Baby trials have been taken up and if dissemination took place who received them and what was the motivation for adoption and which varieties were adopted.

We have also been preparing the PhD work of Ireti Balogun from AbacusBio and the University of Otago, both in New Zealand, who is to do the 1000 minds study within the NextGen survey division. She is about to start here initial fieldwork at the moment of the submission of this report.

The table below shows more information on each student; for a more detailed report from each student on their research activities, please see Appendix 1 Part 4B.

Name	Institution	Nationality	Gender	Research title	Status
Abah, Simon Peter	WACCI	Nigerian	Male	Optimization of Genetic Gain in Cassava Breeding Cycle of Genomic Selection for Storage Root Formation and Shape using High Throughput Screening Systems	Enrolled in first year and successfully engaged in coursework.
Amaefule, Chinedozi	Cornell (Jannink Lab)	Nigerian	Female	Physiochemical characterization and genetic improvement of <i>garri</i> quality of cassava	Enrolled in first year and successfully engaged in coursework.

Ano, Chukwuka	MaRCCI	Nigerian	Male	Evaluation of West African cassava germplasm for resistance to CBSD in Uganda	Enrolled in first year and successfully engaged in coursework.
Bakare, Moshood	Cornell (Jannink Lab)	Nigerian	Male	Estimation of genotype-by-environment interaction in optimizing breeding scheme in cassava	Currently in his second year. IITA is establishing a crossing block for his cassava heterosis project.
Balogun, Irete	University of Otago	Nigerian	Female	New approaches to user-informed design of breeding & evaluation programs for genetic improvement of cassava in low-income countries	
Gwandu, Francisca	MaRCCI	Tanzanian	Female	Root quality and agronomic performance of West African provitamin A cassava in Uganda	Enrolled in first year and successfully engaged in coursework.
Manze, Francis	MaRCCI	Ugandan	Male	Genetic gains and environmental stability of selected traits of Ugandan cassava varieties	Enrolled in first year and successfully engaged in coursework.
Mrema, Emmanuel	WACCI	Tanzanian	Male	Genomic Tools for Resistance to Cassava Brown Streak Disease and Introgression of CBSD Resistant Genes into Farmers Preferred Cassava Varieties	Enrolled in first year and successfully engaged in coursework.
Nandudu, Leah	Cornell (Jannink Lab)	Ugandan	Female	Genetics of cassava brown streak disease	Enrolled in first year and successfully engaged in coursework.
Oluwasanya, Deborah (Ade)	Cornell (Setter Lab)	Nigerian	Female	Regulation of cassava flower development – photosynthate and hormonal factors under field conditions	Currently in Year 4. Expected to graduate May 2020.
Omari, Mikidadi	MaRCCI	Tanzanian	Male	Characterization of selected cassava released varieties in starch content, properties and composition in Lake Zone in Tanzania	Enrolled in first year and successfully engaged in coursework.
Owoade, Durodola	University of Ibadan	Nigerian	Female	Investigating existing seed dealer initiatives and their variety portfolios in Nigeria: social targeting to inform product profiles within breeding	Will complete field research activity between June 1 and July 30, 2019
Sichalwe, Karoline	MaRCCI	Tanzanian	Female	A multi-location evaluation of candidate cassava varieties for field-resistance to cassava brown streak disease (CBSD) in Tanzania	Enrolled in first year and successfully engaged in coursework.
Wembabazi, Enoch	WACCI	Ugandan	Male	Root trait phenotyping using high-throughput technologies	Enrolled in first year and successfully engaged in coursework.

Capacity development (COPP): Community of practice partnership (COPP) built for the application of genomics-assisted cassava breeding across African programs in Ghana, Rwanda, Mozambique, Sierra Leone, Zambia, Malawi, and DR Congo.

We have made big strides on this milestone this period. Project manager Chiedozi Egesi traveled to Sierra Leone from 2-5 July 2018 to integrate the Sierra Leone Agricultural Research Institute (SLARI) into NextGen Cassava under the Community of Practice Partnership (CoPP). Peter Kulakow (IITA) also accompanied him, as well as Alfred Dixon, Director of Partnerships-for-Delivery at IITA and former DG of SLARI.

The NextGen Cassava CoPP was launched at Council for Scientific and Industrial Research--Crop Research Institute (CSIR-CRI) in Kumasi, Ghana, from 6-7 July 2018. The team visited several sites to discuss project activities and possible solutions, interventions and entering points to positively contribute to the cassava breeding programs in the country. All team members expressed hope for fruitful collaboration in the future.

Five cassava breeders from CoPP countries attended the NextGen annual meeting in Kampala in February 2019. There, they participated in workshops and sessions, including one workshop focused on CoPP work planning. The new members are: Isata Kamanda, SLARI; Ruth Prempeh, CSIR; Athanase Nduwumuremyi, Rwanda Agricultural Board (RAB); Martin Chiona, Zambia Agricultural Research Institute (ZARI); and Boni N'ZUE, National Centre for Agronomic Research (CNRA), Côte d'Ivoire.

To support the NextGen Community of Practice Partnership, the IITA Data Management team travelled extensively to several countries in Africa to provide hands-on training use of modern tools for data recording during field phenotyping and use of databases. The workshops took place in Zambia (ZARI), Rwanda (RAB), Ghana (CSIR), Sierra-Leone (SLARI), DRC (INRA) and Ivory Coast (CNRA). A total of 109 technicians and breeders were trained. Key IITA resource people were Afolabi Agbonna and Prasad Peteti. With this development, the NARS CoPP members have transformed their data collection and management procedures to align with modern procedures and have all committed to the usage of Cassavabase for same. Also, NextGen distributed bar code labels to the trainees for field-plot labelling which has been implemented in the partner organizations.

Please see Appendix 2 Part 3 for detailed reports from each of the 5 current CoPP members.

Biotechnology communication: Awareness and outreach campaigns conducted; biotechnology champions identified and supported to organize events; building education nodes in two other NextGen NARS partners; integration of training in formal education systems.

The Uganda Biosciences Information Center (UBIC) continued to support the establishment of an enabling policy environment for effective application and regulation of modern biotechnology products. Activities implemented included organizing monthly NARO strategic meetings, participation in consultations meetings organized by Government MDAs, and organizing seeing-is-believing tours and workshops with for key members of Parliament including the Speaker of Parliament.

UBIC also organized additional nine awareness and outreach activities for farmers' leaders, extension agents, religious, cultural and community leaders and women groups reaching over 600 participants. In partnership with the Cornell Alliance for Science, we conducted three screenings of the [Food Evolution movie](#). We also participated in International and National Agricultural Expos where over 3000 people were reached including farmers, policy makers, civil servants, and students etc.

To support integration of biotechnology teaching in the formal education system, UBIC conducted a week-long training workshop for 30 curriculum specialists and teachers. They carried out our flagship activity of the Annual National Biotechnology Essay-writing Contest whose publicity helped to raise biotech awareness to over 2500 students and educators, and received 202 submissions from 24 secondary schools and 14 tertiary institutions. They also organized university debates in which over 500 students and educators from ten universities participated.

Capacity building for UBIC staff was implemented by facilitating each staff to attend at least one international training program or conference, and at least one national conference or training program. UBIC team continued to train and support regulatory compliance of NARO biotech projects, and biotechnology communication in all NARO Institutes.

UBIC strengthens strategic partnerships with international agencies like ISAAA, Cornell Alliance for Science, University of California, Davis, International Institute for Life Science, European Commission's Joint Research Center, Ghent University and Swedish University of Agricultural Sciences. Regionally, we strengthened partnerships within the African Union, Biosciences for Eastern and Central Africa, AATF to mention a few.

This milestone is for the first two periods of the project only. Future plans for UBIC include:

- Engagements with the Ministry responsible for GER Act (2018) and other relevant Government Ministries, Departments and Agencies;
- More efforts to support the commercialization pathway for the NARO biotech products;
- Continuous engagements of built champions to increase appreciation and support for agri-biotech application for national development; and
- Support developing and piloting a modern biotechnology training manual for secondary schools.

Please see Appendix 1 Part 4C for more details on UBIC's biotech communications activities.

PUBLICATIONS

Title	Journal/Date	Author(s)	Link
Starch Quality Traits of Improved provitamin A Cassava (<i>Manihot esculenta</i> Crantz)	Heliyon; 8 February 2019	Evans Atwijukire, Joseph Ffuna Hawumba, Yona Baguma, Enoch Wembabazi, Williams Esuma, Robert Sezi Kawuki, Ephraim Nuwamanya	https://doi.org/10.1016/j.heliyon.2019.e01215
Genetic Variation and Trait Correlations in an East African Cassava Breeding Population for Genomic Selection	Crop Science; 24 January 2019	Alfred Ozimati, Robert Kawuki, Williams Esuma, Siraj I Kayondo, Anthony Pariyo, Marnin Wolfe, Jean-Luc Jannink	https://doi.org/10.2135/cropsci2018.01.0060
Identification of FT family genes that respond to photoperiod, temperature and genotype in relation to flowering in cassava (<i>Manihot esculenta</i> , Crantz)	Plant Reproduction, 12 December 2018	Oluwabusayo Sarah Adeyemo, Peter T. Hyde, Tim L. Setter	https://doi.org/10.1007/s00497-018-00354-5
A statistical framework for detecting mislabeled and contaminated samples using shallow-depth sequence data	BMC Bioinformatics, 12 December 2018	Ariel W. Chan, Amy L. Williams and Jean-Luc Jannink	https://doi.org/10.1186/s12859-018-2512-8
Training Population Optimization for Prediction of Cassava Brown Streak Disease Resistance in West African Clones	G3: Genes, Genomes, Genetics, 1 December 2018	Alfred Ozimati, Robert Kawuki, Williams Esuma, Ismail Siraj Kayondo, Marnin Wolfe, Roberto Lozano, Ismail Rabbi, Peter Kulakow, Jean-Luc Jannink	https://doi.org/10.1534/g3.118.200710
Gender-based constraints affecting biofortified cassava production, processing and marketing among men and women adopters in Oyo and Benue States, Nigeria	Physiological and Molecular Plant Pathology, Vol. 105; 26 November 2018	Olaosebikan, O., A. Bello, D. Owoade, A. Ogunade, O. Aina, P. Ilona, A. Muheebwa, B. Teeken, P. Iluebbey, P. Kulakow, M. Bakare, E. Parkes	https://doi.org/10.1016/j.pmpp.2018.11.007
Grafting as a strategy to increase flowering of cassava	Scientia Horticulturae 540:544-551; 20 October 2018	Souza LS, Diniz RP, Neves RL, Alves AAC, Oliveira EJ.	https://doi.org/10.1016/j.scienta.2018.06.070
High-Throughput Phenotyping and Genomics-Assisted Breeding for Quality Traits in Cassava	Cornell Theses and Dissertations	Ikeogu, Ugochukwu Nathaniel	https://doi.org/10.7298/X4SN0753
Automating the segmentation of necrotized regions in cassava root images.	In Proceedings of the International Conference on Image Processing, Computer Vision, and Pattern Recognition (IPCVR) (pp. 71-77); August 2018	Ninsiima, F. D., Owomugisha, G. and Mwebaze, E.	https://csce.ucmss.com/cr/books/2018/LFS/CSREA2018/IPC3638.pdf
Genome-Wide Association Study of Resistance to Cassava Green Mite Pest and	Crop Science; 26 July 2018	Lydia Ezenwaka, Pino Del Carpio Dunia, Jean-Luc Jannink, Ismail Rabbi, Eric Danquah, Isaac Asante, Agyemang Danquah,	https://doi.org/10.2135/cropsci2018.01.0024

Related Traits in Cassava		Essie Blay and Chiedozi Egesi	
Cassava Trait Preference of Men and Women Farmers in Nigeria	Economic Botany; 12 July 2018	Olaosebikan Olamide; Haleegoah Joyce; Oladejo Elizabeth; Madu Tessa; Abolore Bello; Parkes Elizabeth; Egesi Chiedozi; Kulakow Peter; Kirscht Holger and Tufan Hale Ann	https://doi.org/10.1007/s12231-018-9421-7
Genomics-assisted breeding in the CGIAR research program on roots, tubers and bananas (RTB)	Agriculture; 22 June 2018	Friedmann, M., A. Asfaw, N.L. Anglin, L.A. Becerra, R. Bhattacharjee, A. Brown, E. Carey, M.E. Ferguson, D. Gemenet, H. Lindqvist-Kreuzer, I. Rabbi, M. Rouard, R. Swennen, and G. Thiele	https://doi.org/10.3390/agriculture8070089
Impact of Mislabeling on Genomic Selection in Cassava Breeding	Crop Science; 21 June 2018	Shiori Yabe, Hiroyoshi Iwata, Jean-Luc Jannink	https://doi.org/10.2135/cropsci2017.07.0442
Functional Genomics to Aid Genomic Prediction Models in Cassava	Cornell Theses and Dissertations	Lozano Gonzalez del Valle, Roberto Jesus	https://doi.org/10.7298/X4FN14D8

2. Project Adjustments

For each outcome or output that is behind schedule or under target, explain what adjustments you are making to get back on track.

At IITA, seedlings from Genomic Selection cycle 4(A) were not genotyped for genomic prediction due to the change in genotyping platform and delays in identifying suitable alternative. However, we implemented phenotype-based selection from these populations and are presently at the Preliminary Yield Evaluation stage.

IITA is on course to reinstate the genomic selection cycles rebuilding our training population by generating phenotypic and genotypic data. More than 1600 seedlings from cycle 4 were cloned in 2018 and planted in three clonal evaluation trials. These seedlings will be genotyped using the new high-density genotyping platform, DARTseq along with 2500 seedlings that were cloned for stem multiplications. The idea is that we will use the data from the phenotyped individuals in the training population to predict the unphenotyped individuals and thereby increase population size.

During the present reporting period, the IITA cassava breeding program was fortunate to undergo the Breeding Program Assessment Tool process that was administered by a team led by researchers from Queensland University, Australia. They have pointed out areas of strength and areas that need improvement. We have shared the report with NextGen breeders in order to address the issues. We will be seeking the support of the Excellence in Breeding platform through development of a breeding program improvement plan and initial development of variety replacement strategies.

At CIAT, shipment of botanical seed and *in vitro* plantlets is lagging behind. All the material is ready for shipment and just pending on the required paperwork to be completed. The CBSD immune plants were transplanted (after hardening from the *in vitro* status) into the field under RLT. Plants from several accessions branched but did not produce flowers. It is possible that because plantlets were transplanted "late" in their phenological development (e.g. red light was not applied at the sprouting), the beneficial effects of RLT were not achieved. Plants will be ratooned in May.

At the Makerere AIR lab, the version of Fieldbook App with integrated CBSD app is not yet deployed. More testing is required with the FieldBook team. Appropriate transmission of images from the field not yet implemented. Also dependent on BrAPI specification implementation. In the second year, AIR lab members plan to make a visit to the FieldBook team to test and deploy their application within FieldBook. They also plan to make a technical visit to BTI in the second year to meet with the Cassavabase team to work on the implementation for the integration with Cassavabase.

The outcome under Survey division relating to screening NextGen populations with HTTP outputs from RTBfoods was written when RTBfoods was still in conceptualization stage. At the time we had anticipated HTTP deployment on NextGen populations would be coordinated through NextGen. RTBfoods has hence taken on a more leading role to coordinate HTTP deployment across all crops through WP4 (breeding). HTTP training and population screening has there been coordinated through RTBfoods. We will keep this outcome for Period 2 as we fine-tune, but we will need to determine whether this outcome should be removed from NextGen's results framework.

3. Feedback for the Foundation

Provide one to three ways the foundation has successfully enabled your work so far. Provide one to three ways the foundation can improve.

We continue to receive wonderful support from the Foundation, and are very grateful. Our feedback remains very similar to the feedback we offered in our last report, which was less than a year ago, at the end of Phase 1 of the project.

One of our most considerable (and consistent) areas of support from the Foundation is their help in giving us access to external resources and stakeholders from both private and public sectors. We continue to benefit from the expertise of our external project advisory committee (EPAC), as well as through exchanges with the private sector (Syngenta and Corteva) on best practices that can be implemented in public breeding organizations, especially in relation to improving the quality of our field experiments and in reduction of error variances. Also, ensuring that our linkages with the Excellence in Breeding platform guaranteed productive working relationships with each of the breeding partners has resulted in accountable breeding programs.

The Foundation continues to support several interrelated cassava projects, offering us further benefit for our research and growing our network of cassava researchers. We look forward to continued collaboration with these projects, such as the African Cassava Whitefly Project, RTBfoods, HarvestPlus, BASICS, WAVE, ACAI, Excellence in Breeding, etc.

The support of the NextGen Cassava Program Officer Jim Lorenzen continues to be extraordinary. He remains available for regular discussions and troubleshooting, and always offers an open mind and a fair and balanced perspective. His presence at our annual meetings is extremely valuable in helping us always come back to the big picture, reminding us why we do the work we do, and the impact it is having. We continue to be very grateful for Jim's support and expertise, which have continued to contribute immensely to the success of the project, and has helped to steward us through the transition to Phase 2.

Currently, we feel comfortable with the relationship we have had with the Foundation in project administration. In our last report, we mentioned seeking further engagement with EiB to enable Cassavabase to become the universal database for cassava; the Foundation has supported us in our EiB interactions. We still continue to push for more support for long-term sustainability in capacity building/student training in African breeding programs. We continue to have good interactions with Flutra Loun on financial matters, and hope she will continue to support us going forward; we also appreciate the support we receive from Amy Pope.