

## 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.

### General Information

Investment Title	AfricaYam: Enhancing Yam Breeding for Increased Productivity and Improved Quality in West Africa		
Grantee/Vendor	International Institute of Tropical Agriculture		
Primary Contact	Patrick Adebola	Investment Start Date	14 October 2014
Feedback Contact <sup>1</sup>	Patrick Adebola	Investment End Date	30 April 2020
Feedback Email <sup>1</sup>	p.adebola@cgiar.org	Reporting Period Start Date	1 February 2018
Program Officer	Jim Lorenzen	Reporting Period End Date	31 January 2019
Program Coordinator	Amy Pope	Reporting Due Date	15 March 2019
Investment Total	US\$13,500,000.00	Opportunity/Contract ID	OPP1052998
Scheduled Payment Amount (If applicable)	US\$1,524,054.00		

<sup>1</sup> Feedback Contact/Email: The full name and email of the contact whom foundation staff queries for various surveys.

### Submission Information

By submitting this report, I declare that I am authorized to certify, on behalf of the grantee or vendor identified on page 1, that I have examined the following statements and related attachments, and that to the best of my knowledge, they are true, correct and complete. I hereby also confirm that the grantee or vendor identified on page 1 has complied with all of the terms and conditions of the Grant Agreement or Contract for Services, as applicable, including but not limited to the clauses contained therein regarding Use of Funds, Anti-Terrorism, Subgrants and Subcontracts, and Regulated Activities.

Date Submitted	[15 03 2019]	Submitted by Contact Name	Dr Allan Liavoga
		Submitted by Contact Title	Head, Project Dev. & Admin Unit
		Submitted by Contact Email	A.liavoga@cgiar.org
		Submitted by Contact Phone	+2348039784491

## 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.

#### Executive Summary

Training and capacity building of national partners continued in Year 4. There were several trainings for technicians and exchange visits among the national partners during the reporting year. IITA breeding program received breeders from most of the national partners as part of the cross-partner exchange visits and we shared experiences on crossing, screen house seedling nursery management, field tuber family evaluation, early clonal generation nursery assessment, and advanced clonal performance evaluation and selection. The Boyce Thompson Institute (BTI) participated and gave workshops at Abidjan in March 2018 and also twice at Abuja in June and November 2019. Representatives from all breeding programs attended the trainings. The team at BTI continued to work closely with breeding stations

to ensure adequate integration of YamBase database in their daily routine activities. JIRCAS also conducted training for IITA staff for tuber quality evaluation methodology and the evaluation of virus infestation rate.

During the reporting year, CNRA acquired one electronic microscope, one fridge, three weighing scales, and one laminar flow which were installed in their pathology laboratory. The disease phenotyping team at IITA purchased an additional ipad for increased efficiency in the DLA estimate scores. Infrastructural challenges however, still remain. For example, CSRI-SARI, CNRA, and UAC had limited storage facilities for the increasing number of planting materials generated from the program and limited screen house space for the large number of seedlings generated.

The AfricaYam Website was updated regularly and improved during the year and the Yam Community of Practice (YCoP) was very active with number of registered and active users growing. This has encouraged information sharing and discussion and has helped strengthen YCoP. The Annual Progress Review and Work Planning Meeting was held at Tiama Hotel Abidjan, Cote d'Ivoire, from 26 February to 2 March 2018. The project's Technical Advisory Committee (TAC) also met during the period and valuable suggestions were provided to the Program Management to ensure project milestones and outcomes are met. A total of 36 students (10 PhD, 11 MSc, and 15 BSc) have been engaged and are conducting research on various aspects of the project in the national programs and at IITA. Through these studentships, the yam breeding programs of national institutes are being strengthened and equipped with additional human capacity. Several manuscripts on the projects were prepared and are at various stages of publication. A few staff changes occurred during the reporting period: Dr David Dekoeyer left IITA and Dr Asrat Amele was appointed to replace him as IITA and AfricaYam breeder with 40% time on the project. Dr Agre Paterne was also appointed as an Associate Scientist (molecular breeder) and the Project Administrator, Mr Olukotun Olubunmi resumed office on 1 March 2018. The project also had an internal audit review and the outcome was rated very good.

Significant progress was made in developing genomic resources and populations for genetic studies in Year 4. Both bi-parental and GWAS populations were successfully phenotyped and genotyped as planned. Preliminary trait-marker association analysis identified putative regions of the yam genome that explains the variation in plant sex and other relevant traits. In addition, new breeding populations were developed, and the existing populations were advanced to the next breeding stages. With the variety development pipeline, different stages of breeding trials were executed in the 2018 cropping season. The trials in 2018 composed of crossing block (both controlled and open polycrossing block), seedling progenies, tuber family evaluation, clonal nursery evaluation, preliminary performance trials, advanced performance trials, and multi-location yield trials. The program executed different stage trials at various sites in Nigeria: Abuja, Ago-Owu, Ibadan, Ikenne, Nassarawa, and Ubiaja. In addition to the on-station testing, on-farm variety verification trials both in *D. rotundata* and *D. alata* were executed over 15 sites across Nigeria in collaboration with NRCRI-Umudike. The national variety release committee technical team visited the on-farm trials aimed at the release of new series of improved yam varieties for commercial production in Nigeria. All trials were harvested and data from breeding and genetic study trials were uploaded to the YamBase.

In 2018, disease phenotyping activities focused on the validation and the use of the newly developed screening protocols. The Detached Leaf Assay (DLA) was used for yam anthracnose disease (YAD) phenotyping of *D. alata* populations (TDa 1401 mapping population). The 'ESTIMATE' app was developed using YAD standard area diagrams (SAD) for image-based phenotyping in the field and DLA. This app reduces time for assessment and improves accuracy of phenotyping. The tool is validated and will be disseminated to partners in 2019. A method for mechanical inoculation of YMV was also perfected offering 95% transmission rate. This method has opened new opportunity for accurate YMV phenotyping of *D. rotundata* breeding populations.

The features on the YamBase were again improved with the addition of 23 more tools and its use within the yam breeding team continue to increase during the year. The database has been updated and now counts over 54 000 accessions with over 1500 genotypes, seven breeding programs, 197 assayed traits, about 414,000 phenotype scores, and over 325 trials. Website functionality has been adapted to breeder's input. The database has contributed to harmonizing data and standardizing procedures across breeding programs. It has also enhanced collaboration across partners and supports YCoP. [These interactions with partner projects and other programmes are already having positive impact. Yam product profiles and yam improvement plan had already been developed following recommendations from BPAT and EiB. Going forward, YIIFSWA will be a key partner in rolling out released varieties and also getting feedback from target beneficiaries.

The milestones reported are arranged by Primary Outcomes. A more detail activity report by work package, is attached as Appendix 1.

## PRIMARY OUTCOME 1

### Training and exchange visits

Training and capacity building of national partners continued in Year 4. BTI participated and gave a workshop at the annual review meeting of the project held at Abidjan in March 2018. A member of the YamBase development team visited the IITA yam breeding station in Abuja mid-June for a week-long training and workshop session.

**Commented [JL1]:** Given that this investment is supporting IITA's breeding, including interactions with BPAT, EiB, RTBFoods, YIIFSWA, etc., it would be helpful to acknowledge those interactions and describe where they are intersecting with the project. Action plan for BPAT, product profiles for EiB, etc. should be included and results framework adjusted to allow for those. What is action plan to identify new varieties that will be highly demanded and that could be justifiably included in YIIFSWA multiplication plans? How can YIIFSWA be used as an additional source of market feedback on advanced selections or recently-released varieties? What food quality traits need to be included in product profiles to have highly-successful varieties?

**Response:** Yes. These interactions with partner projects and other programmes are happening and already having positive impact. Yam product profiles and yam improvement plan had already been developed following recommendations from BPAT and EiB. Going forward, YIIFSWA will be a key partner in rolling out released varieties and also getting feedback from target beneficiaries. We will update our result framework to reflect all these outcomes. The major quality traits that are included in yam product profile are: For pounded yam – White color, high firmness, good aroma, high pectin content, smooth texture, stretchability, consistency (temperature non-dependent poundability), Dry matter (≥ 25%), Oxidization(No oxidation after 180 minutes, better or comparable quality with best check in the market.

For boiled yam – Fast cooking, sweet taste, soft slice, good flavor, Dry matter, Tuber oxidation, Tuber shape (Cylindrical or round), , No after cooking darkening, better or comparable quality with best check in market.

More details are available in the attached yam product profile.

Between 12 and 17 November, another week-long YamBase hands-on training was held at a central location (Abuja, Nigeria) with a total of 27 participants from all breeding programs in attendance. BTI hosted Dr Agre Partene, a postdoc with the IITA, Ibadan breeding station in October 2018 to conduct research relevant to the project. The team at BTI has continued to work closely with breeding stations to ensure adequate integration of YamBase database in their daily routine activities.

In Universite d'Abomey-Calavi (UAC), one technician was trained for 25 days in the tissue culture laboratory of the Department of Genetics and Biotechnology of Calavi (UAC Main Campus) in September 2018. Several exchange visits among partners happened during the reporting period. Patrick Adebola and Asrat Amele visited most of the partner institutions to assess and monitor the implementation of project activities. Dr Emmanuel Otoo from CSRI-CRI visited IITA, Abuja station from 24 August to 1 September 2018. Dr Adebola and the newly appointed project administrator visited the National Root Crops Research Institute (NRCRI) and the Ebonyi State University (EBSU) during 24–26 July 2019. Their visit was to ascertain the level of activities going on among partner programs, the trials, reporting systems, challenges, and proposed solutions. He also emphasized the need for publications and scientific contributions as outcomes of the project. Professor Alexandre Dansi and Dr Innocent Dossou Aminon from UAC attended the Excellence in Breeding Program meeting organized by the yam team of IITA on 5 November 2018.

Several trainings were conducted for IITA staff by JIRCAS scientists, especially for the tuber quality evaluation technologies. JIRCAS also conducted training for IITA staff in partnership with the IITA Virology Unit, on the time-course evaluation of the virus infestation rate of two mapping populations to understand the modernity and trends of virus infestation in field operations.

#### Yam Community of Practice (YCoP) and Annual Meeting

The AfricaYam Website was updated regularly and improved during the year and the YCoP is very active with the number of registered and active users growing. This has encouraged information sharing and discussion, and has helped strengthen YCoP. Project partners from all participating countries and other institutions attended the Annual Progress Review and Work Planning Meeting held at Tiam Hotel, Abidjan, Cote d'Ivoire from 26 February to 1 March 2018. The project's Technical Advisory Committee (TAC) also met during the period and valuable suggestions were provided to the program management to ensure project milestones and outcomes are met. The activity calendar for the 2018/2019 season was completed for all partners. The data for 2018 collected on all trials were submitted and curated on the YamBase. The 2019 trial layouts and field plans were also provided and uploaded on YamBase prior to planting. The partners effectively used the standard protocols developed by IITA (Asrat et al. 2016) for the implementation of trials. This included protocols for land preparation, seed preparation, planting, fertilization, data collection, weed and pest control, harvest, and storage.

#### Students and Publications

More students joined the project during the reporting year. A total of 36 students (10 PhD, 11 MSc, and 15 BSc) working on various aspects of the project are now involved with the national partners and IITA. In Centre National de Recherche Agronomique de Cote d'Ivoire (CNRA), one master's student joined the project in December 2018 with focus on the virus screening of the hybrids at early stage. A total of three PhD students are now working actively on the project in CNRA. One of the students, Adou Emmanuel, spent six months at CIRAD Montpellier for a training on the NIRS (February to July 2018). His stay was financially supported by the RTBfoods project. He was able to screen two populations of *D. alata* hybrids of CIRAD. The results are reported by CIRAD. Another PhD student, Bakayoko Yacouba, got a fellowship from INRA Guadeloupe. He stayed in Guadeloupe from October to December 2018 to conduct research on yam virus diseases. He was also able to screen 34 accessions of *D. rotundata* of the CNRA yam germplasm and 210 hybrids. In EBSU, two MSc and four undergraduate students joined the project. One of the MSc students was engaged for genetic diversity studies of about 250 yam accessions collected from Ebonyi State and beyond. Presently the student is in IITA, Ibadan for DNA extraction

**Commented [JL2]:** These are not emphasized from our side - efficient breeding and development of market-preferred varieties that achieve significant market share is the desired outcome

**Response:** This is noted.

**Commented [JL3]:** Where are these details? Were these "clean" populations when planted, or was there already some virus infection at the time of planting? What were check genotypes and infection status at planting?

**JIRCAS response:** The detailed information should be reported by Virology unit for multiple year evaluation. The accessions of two mapping populations were clean enough at year 2013-2014 since they are newly derived from botanical seed and kept in the screen house for seed tuber development. Since then, through field trial and regeneration, virus infestation rate has been increasing, and we invite virology unit to evaluate virus infestation status in 2016 and 2018. We believe those data provides insight for field virus infestation modality.

prior to genotyping using the BecA-ILRI platform. Two additional BSc students joined the project at UAC during the year. In SARI, two PhD and one BSc students join the project during the year.

Mueller Lab, gave a presentation at the Yam Genomic Workshop during the 2019 Plant and Animal Genome Conference, held at San Diego, California. It was titled “YamBase, a comprehensive digital ecosystem for yam breeding”, and captures the concept of YamBase as a comprehensive digital ecosystem platform with computational infrastructure and seamless integration to routine yam breeding pipeline activities. In CIRAD, a poster (Ehounou et al. 2018) titled “Breeding for Improved Tuber Quality in Yam *Dioscorea alata* L.” was presented at the 18<sup>th</sup> Triennial Symposium of ISTRC, Cali, Colombia, 22–25 October 2018. UAC also presented a poster (Dossou-Aminon et al. 2018) entitled “Pollen viability and storability of yam (*Dioscorea rotundata*) cultivars involved in breeding for food security in West Africa” at the same conference. A publication (Fabien et al. 2019) entitled “A reference high-density genetic map of greater yam (*Dioscorea alata* L.” submitted to TAG on 7 November 2018 was accepted for publication in January 2019. Three technical papers were published, and six manuscripts are at various stages of publication in CRNA. Dr Amani Michel Kouakou, yam breeder from CNRA, attended the 2<sup>nd</sup> International Yam Crop Science Workshop at the University of Agriculture in Tokyo, Japan, 8 to 11 March. He made a presentation entitled: “Yam breeding in Côte d’Ivoire: Progress and challenges”. Dr Amani also attended the annual meeting of the yam system for improved food security in West Africa (YAMSYS) project at Yamoussoukro, Côte d’Ivoire on 3 February 2018. During this meeting, the participants decided to create a yam organization for Côte d’Ivoire and Burkina Faso as a forum to advocate and lobby the countries’ policy makers to have a strategy for yam development. The event is scheduled take place in October 2019 at Abidjan. EBSU reported the publication of two conference proceedings during the reporting year. Also, in UAC, four manuscripts are at various stages of publication.

#### Infrastructure and Equipment in Support of Yam Breeding

During the reporting year, CNRA requested and was given approval to acquired one electronic microscope, one refrigerator, three weighing scales, and one laminar flow, which have been installed in the pathology laboratory. The disease phenotyping team at IITA purchased an additional iPad for increased efficiency in the DLA estimate scores. No other major infrastructural improvements were made during the reporting year. Infrastructural challenges however, still remain. For example, CSRI-SARI, CNRA, and UAC had limited storage facilities for the increasing number of planting materials generated from the program and there is limited screen house space for the large number of seedlings generated.

#### Project Management

The AfricaYam financial year now effectively runs from 1 February to 31 January each year to coincide with the yam cropping cycle. A few staff changes occurred during the reporting period. Dr David Dekoeyer left IITA and the project as yam breeder at the end of his contract term in April 2018. Dr Asrat Amele was appointed to replace Dr Dekoeyer as IITA and AfricaYam breeder with 40% time on the project. Dr Agre Paterne was appointed as an Associate Scientist (molecular breeder) during the year. He was previously a postdoc on the project. A new Project Administrator, Mr Olukotun Olubunmi resumed office on 1 March 2018. The project also had an internal audit review and the outcome was rated very good.

#### PRIMARY OUTCOME 2

##### Phenotyping of bi-parental mapping populations of *D. rotundata* and *D. alata*

At IITA, two bi-parental mapping populations (one each for *D. rotundata* and *D. alata*) were phenotyped for two consecutive cropping seasons: 2017 and 2018. The biparental mapping populations were coded as TDr1402 (TDr97/00917 × TDr99/02789) and TDa1401 (TDa05/00015 × TDa99/00048). The progenies of the mapping populations planted for phenotyping in 2017 included 215 clones of TDr1402 (*D. rotundata*) and 247 clones for TDa1401 (*D. alata*). We experienced loss of progenies during the field phenotyping due to non-sprouting, an early foliar disease infection shortly after sprouting, and failure to produce viable tubers at harvest (non-tuberization). In the 2018 cropping season, new progenies of the same crosses were added to those that produced tubers in 2017

**Commented [JL4]:** Could I get a copy of these two posters?

**Response:** Posters already sent to Jim.

to boost the population size to an acceptable level for the genetic study. The partial replicated field design was used for both seasons field trials.

#### ***D. alata* mapping population (TDa1401)**

The *D. alata* mapping population comprised 207 progenies (75 new progenies which were initially not part of the 2017 phenotyping experiment in addition to the 132 progenies that produced tubers in 2017) in the 2018 phenotyping. The progenies were randomly assigned to three replications based on the availability of tubers (planting materials). In all, 25 progenies were featured only in single replicate while the others partially replicated (52 progenies were replicated twice and 130 replicated three times). Of the newly assembled 207 progenies planted in the 2018 season, 31 were lost due to non-sprouting and an early foliar disease infection (probably anthracnose) shortly after sprouting. A total of 176 progenies survived and produced tubers at harvest. Data was collected (2017 and 2018) on days to sprouting, number of stems, stem diameter, plant vigor, canopy architecture, anthracnose and yam mosaic virus disease incidence and severity (for 2 months and at 2 weeks interval), presence of bulbils, senescence class, days to flowering, plant sex and flowering intensity, and yield and yield components including number of plants harvested, number of tubers, tuber length and width, and tuber weight (categorized as big, medium, and small). Data was collected based on the standard operating protocol for yam variety performance evaluation trial (Asfaw 2016) and the yam trait ontology available on YamBase (<https://yambase.org/>). In addition, the 2018 plants were challenged with artificial inoculation of a highly virulent strain of the yam anthracnose disease causing organism, *Colletotrichum gloeosporioides*, under laboratory condition using the detached leaf assay (DLA) technique. Two leaf samples were collected from each progeny for inoculation. The DLA data was collected on the percentage leaf surface diseased (% severity) at 7, 14, and 21 days after inoculation and the mean computed (for details on methodology, refer to “high throughput phenotyping for anthracnose” section). The field resistance under natural infestation and true resistance under artificial challenge with pathogen were compared to see the consistency of the phenotypic response of the progenies for exposure to natural infestation and artificial inoculation. There was no significant difference between the progenies of TDa1401 mapping population for most of the traits assessed in the 2017 growing season except for stem diameter, tuber yield, and cracks on tuber surface that showed significant variation in the population. Details of the results are shown in Appendix 1.

In the 2018 season, a total of 146 progenies of TDa1401 were used for the detached leaf assay. There were significant differences among the progenies for mean YAD score (DLA). The means of the top 30 performing progenies are presented in Appendix 1. Of the 65 progenies that gave mean YAD score of 2.0 (DLA), only 1 had a corresponding 2.0 mean YAD score in the field. In all, 5, 47, and 11 progenies had YAD score of 3.0, 4.0, and 5.0 in the field, respectively, as against their YAD score of 2.0 in DLA. In general, the misclassification of progenies to the wrong category of susceptibility or resistance to YAD by the two methods (DLA and field scoring) mainly occurred at the top score of 2.0 for DLA and the corresponding score in the field. Of the 12 progenies with YAD score of 5.0 in DLA, 1, 2, and 8 had corresponding YAD scores of 5.0, 3.0, and 4.0 in the field. Results of the simple linear regression analysis of mean YAD score measured in the field and DLA are presented in Appendix 1. There was no significant relationship between the response of progenies for reaction to yam anthracnose under natural field infestation and controlled artificial inoculation with detached leaf assay technique. This indicates that field performance for YAD may not be predicted based on DLA scores and vice versa.

#### ***D. rotundata* mapping population (TDr1402)**

For the *D. rotundata* population, 197 progenies were phenotyped in the 2017 cropping season of which 102 were raised from the vine cuttings while 95 progenies were raised using seed tubers. The progenies were planted in a partial replicated trial. Twenty-one agro-morphological (qualitative and quantitative) traits were recorded using the yam ontology crop as reference (<https://yambase.org/tools/onto/>). Like with the *alata*, some progenies failed to produce tubers. Seventy-five progenies out of the 92 tuber-based planting survived in the field till harvesting, however, only 64 produced tubers at harvest. Results from the analysis of variance showed significant differences among the accessions for plant vigor and number of stems, while no significant difference was observed among the accessions for mean YAD and mean YMV scores. YMV scores ranged from 2.0 to 4.0 with a mean of 3.0.

**Commented [JL5]:** What does this say about how to multiply and maintain plants of mapping populations? Will SAH help? If you plant 100 genotypes in SAH trays, what percentage will make minitubers? This is a serious issue going forward - how will you manage populations for which you will need multi-location, multi-year data?

**Response:** Multiplication and maintenance of mapping population is the main challenge. This is because of the seedling progeny management strategy we followed while generating this mapping population. With the intention to obtain sufficient planting materials for the field phenotyping, the seedling progenies were multiplied using vertical sack vine propagation method that complicated combining plantlets or tubers coming from vines and mother seedlings. This resulted in different physiological age and quality of planting materials for field planting in 2017. We combined minitubers coming from seedlings and vine cuttings as planting materials for 2017 planting. Seedling weakness and death could also be attributed to deleterious mutation. What we can learn from this is proper planning for multiplication and maintenance of mapping population. Yes SAH will help and seems promising for planting materials multiplication and maintenance. We are testing the potential of SAH with seedling progenies in 2019. In SAH tray over 80% plantlets produce minitubers. We will optimize the SAH for mapping population multiplication and maintenance to scale phenotyping at multi-site and multiple season.

**Commented [JL6]:** What can you learn from this in terms of required plot size, seed uniformity, etc.? Are you tracking seed details (position on mother tuber, size or weight, etc.)? Are there any other seed physiology issues that might affect performance?

**Response:** The main issue here was heterogeneity of planting materials of the same progenies. Within plot variability was so high as planting materials combined from minitubers and vine cuttings. Yes it possible to track the seed or planting materials source like position on mother tuber, size and weight too.

**Commented [JL7]:** So, what are the immediate conclusions from this experience?

**Response:** The pathogen dynamics on the field might be changing. The pathogen collection for the laboratory testing might need to be updated. This indicates the relevance of field resistance/tolerance testing on regular basis to validate the laboratory tests.

**Commented [JL8]:** When there is insufficient planting material for a population, is it better to have this type of mixed seed type planting material or would it be better to use all one type, e.g. vine cuttings? When analyzing phenotyping results, was seed type used as a co-variate in the analysis?

**Response:** It is not advisable to have this mixed seed type for planting as per our observation.



Six of the 75 progenies that survived had mean YMV scores of 2.0, 68 accessions recorded YMV scores of 3.0, while 1 accession had mean YMV score of 4.0. All the 75 accessions had mean YAD scores of 2.0. Number of stems ranged from 1.0 to 3.0 with a mean of 1.0. Plant vigor ranged from 1.0 to 3.0 with a mean of 2.0. Total number of tubers per plot ranged from 1.0 to 5.0, while total tuber weight per plot ranged from 0.02 to 2.8 kg. Tubers were categorized as small, medium, and big and their respective length, width, and weight recorded. Data was also collected on tuber hairiness, spines, and appearance. Means of some selected traits of the top 30 accessions for YMV score are presented in Appendix 1 while the full data for all accessions and traits are uploaded in YamBase. The field trials raised from tubers and vine cutting as planting material were treated as a separate experiment. All the 102 progenies planted from vine cuttings survived in the field but only 94 produced tubers. Similar to what was observed in the plants established from the tubers, significant differences were observed among the accessions for plant vigor and stem number while mean YAD and YMV scores showed no significant differences. Details are as shown in Appendix 1. The full data for all accessions and traits has been uploaded in YamBase. In 2018, field phenotyping was accomplished with 151 progenies and data will soon be analyzed and uploaded to the YamBase.

Bi-parental mapping populations of *D. alata* planted at CIRAD (Guadeloupe) for anthracnose and quality traits were also phenotyped. At harvesting, the tubers of each trial (2 trials  $\times$  314 genotypes  $\times$  9 replicates) were placed in a net bag, taken into a yam store, and weighed (yield). The following visual assessments were made on all tubers: tube size (length and width), regularity of tuber shape (scored to 0 = high to 2 = low scale), hairiness (scored 0–1–2, 0 = absence, 1 = low, 2 = high), and skin rough (scored 0–1, 0 = smooth, 1 = rough). Four tubers were cut longitudinally for each genotype and replicate. The flesh color and the phenomena of oxidation of flesh were noted at three different parts of the tubers (head, middle, tail). The central part of the tubers (about 200 g) were peeled with a knife, washed, and used for the preparation of chips (diameter 10 mm, height 20 mm). These samples were used for NIRS analysis. Chips were dried at 60 °C for 48 h and milled into flour using a stainless-steel grinder. A total of 2400 samples were prepared. The granules were homogenized using a sieve of diameter 200  $\mu$ m. This method will be used to predict the content of the major constituents (starch, sugar, proteins) and textural characteristics on progenies. NIRS calibration was conducted in the context of another project (RTBFOODS) and in collaboration with the Food Processing Laboratory of INRA. Measurements of all spectra over the wavelength range of 400–2500 nm were made using an NIRSSystems 6500 spectrophotometer. An ANOVA was performed to evaluate the variance of the traits in both populations and to determine if there were significant differences among the progenies. P-values for the genotype effect were significant for all traits ( $P < 0.05$ ) except for hairiness in population B. The highest coefficient variation (93.99%) was obtained for tuber size (ratio length/width) in population B (Details in Appendix 1). Both *D. alata* bi-parental populations and their progenies were again planted in replicated trials in 2018 to collect second year phenotyping data for yield and different quality traits. The trial consists of 18 replicates per genotype (2 trials  $\times$  9 replicates per genotype). Harvesting and second cycle of phenotyping was initiated in January 2019 and is in process.

JIRCAS conducted the four-year field trial for the 1<sup>st</sup> batch of non-redundant set (2015–2017) and whole non-redundant (2018–2019) to generate phenotyping data sets on various agronomic and tuber quality traits. The trial was conducted at the experimental field in IITA HQ, with adequate field rotations to suppress the problems of continuous cropping. The 1<sup>st</sup> batch of the non-redundant set consisted of 119 germplasm lines of *D. rotundata*, including 102 germplasm lines of *D. rotundata* Diversity Research Sets (DrDRS) generated by JIRCAS through EDITS-yam project (2011–current), and 17 breeding lines/local materials. The 2<sup>nd</sup> batch of the non-redundant set which was selected collaboratively by IITA and JIRCAS in 2017, consisted of 85 germplasm lines, 99 breeding lines, and 19 local materials. The 1<sup>st</sup> batch of the non-redundant set was used throughout four-year activities, and the 2<sup>nd</sup> batch of the non-redundant set was added in the 3<sup>rd</sup> and 4<sup>th</sup> year activities to complete the evaluation of the whole set of non-redundant lines (a total 319 lines).

For agronomic traits, flowering date/sex, senescence and maturity date, shoot and tuber numbers, tuber total yield and average size of tuber were obtained per plot, and selected harvested tubers from 1<sup>st</sup> and 2<sup>nd</sup> year activities were used for the evaluation of starch characteristic, tuber flesh color, and oxidation level. There was no tuber quality data obtained from the harvests of 3<sup>rd</sup> year activities to avoid destructive analysis due to limited quantity

**Commented [JL9]:** This is a higher percentage than observed in those planted from tubers? Why?

**Response:** Might be this is due to the physiological quality of the minitubers used for this planting.

of tubers for Year 4 planting, and the evaluation of tuber quality for 4<sup>th</sup> year activity is now ongoing. Throughout the four-year trials, wide genetic diversity in various agronomic traits was observed among the lines of both the 1<sup>st</sup> and 2<sup>nd</sup> batch of the non-redundant sets. Flowering date ranged from 20 July to 8 October corresponding to 200 and 280 of DOY. The observed variations in agronomic traits were similar in both the 1<sup>st</sup> and 2<sup>nd</sup> batches of the non-redundant sets, while the 2<sup>nd</sup> batch of the non-redundant set tended to show higher mean values of tuber yield and average tuber size, which may be induced by the greater proportion (49%) of breeding lines. See details in Appendix 1. As showed in various studies, inconsistency of agronomic traits was also observed, but two traits, date of senescence onset and date of maturity, showed a good level of consistency among replicated years. Even though the agronomic traits showed great variance in the three years (2014–2016), interestingly, there was a clear correlation between the numbers of stem and tuber identified. The tuber quality traits (tuber color and starch property) were relatively stable in the two years (2015 and 2016).

Similar to agronomic traits, the 1<sup>st</sup> batch of the non-redundant set also showed wide genetic diversity in tuber quality traits. Using the evaluation tools developed in EDITS-yam project, the pasting properties of extracted starch were characterized and showed great variance among 119 lines which exceed the difference between two species *D. rotundata* and *D. cayenensis*. Using three parameters (peak, trough, and final viscosity pasting property), the genotypes were classified in six groups, and the typical RVA pattern for each group was identified. On the characteristic of pasting property, especially on final viscosity, the significant contribution of combined amylose and phosphorus contents was also identified. The tuber fresh color and oxidization characteristic were also evaluated and currently an adaptation of the new method of image analysis is attempted for quicker and large-scale evaluation. Also, several potential materials were nominated for the development of new mapping populations targeting the further study of tuber color and oxidization characteristics.

Based on the suggestion given by Technical Advisory Committee in the last annual meeting in Abidjan (2018 March), the evaluation of the pasting property of freeze-dried yam flour was conducted. Low correlation was observed between the pasting behaviors of extracted yam starch and those of freeze-dried yam flours with amylase inhibition suggesting that the complexity of the factors related to the pasting behavior of freeze-dried yam flour, consisted of starch, fibers, inherent enzymatic activities, among others. This result suggests importance of evaluation using freeze-dried yam flours which would be linked to the quality of yam product, though the data may be difficult to utilize for genotypic analysis at the current stage due to the complexity of the factors involved. All obtained phenotyping data were uploaded into the Yam-base, though original data of some traits which have not been well categorized in the database was shared with the IITA yam breeding unit for their immediate use. To harmonize the categorization, further discussion is required. The results were presented at several conferences such as those of ISTRC 2017, 2018 Japanese Society of Breeding, and 2018 Crop Science Society of Japan, and the manuscripts for scientific publication are being prepared.

Field trials for two mapping populations, MP-EDITS1 (2015–2017) and MP-EDITS 2 (2015–2018), generated by the EDITS-yam project of JIRCAS, were conducted to generate phenotyping data sets for various agronomic traits. The trials were conducted in complete randomized design with three replicates at the experimental field in IITA HQ, with adequate field rotations to suppress the problem of continuous cropping. In both mapping populations, wide genetic diversity in various agronomic traits were observed and it was identified that the level of variation was similar to or even larger than that of whole set of non-redundant lines. Several traits, such as flower sex, stem member, and tuber number were relatively consistent among the years, while flowering date, tuber yield, and average size of tuber showed significant influence of growth condition of each year. The obtained phenotyping data was uploaded to Yam-base.

To harmonize the activities of IITA (breeding), IBRC (genotyping), and JIRCAS (phenotyping), JIRCAS coordinated the active material and information exchange among the scientists participating in the Africa Yam project. For the genotyping activity of IBRC, JIRCAS has prepared and provided leaf samples of non-redundant

**Commented [JL10]:** Is anything known about the cooking quality of these 6 groups?

**JIRCAS Response:** We did not have the date for cooking quality itself, and it will be difficult to correlate cooking quality and starch pasting behavior due to the various factors affecting pasting property of fresh yam as described in the next section.

However, we try to include some market varieties collected from South-western Nigeria, and some high value varieties are only distributed in two clusters.

**Commented [JL11]:** What is the chemical nature of the phosphorus? Is it incorporated in the starch as G-6-P and/or G-3-P?

**JIRCAS Response:** Our analysis of P is only total P contents and not evaluated forms of P in the starch.

**Commented [JL12]:** Less correlation compared with which other comparison?

**JIRCAS Response:** We should say “low correlation” rather than “less correlation”. No comparison was made.

lines and two mapping populations (MP-EDITS2) for DNA extraction and further genotyping activities at IBRC. All phenotyping data sets have been shared with IBRC for the analysis, and JIRCAS scientist(s) regularly visit IBRC for detailed discussion and information sharing with IBRC scientists. The utilization of both phenotyping and genotyping data generated by the two institutes for genetic analysis will be reported by IBRC.

The maintenance and management of generated materials are two of the constraints of clonal crops, including yam due to its large plant size and propagation modality. However, to enhance yam research, the sharing of the common/standard plant materials is quite important. In view of this, JIRCAS facilitated the maintenance and sharing of the materials of the 1<sup>st</sup> batch of the non-redundant set and the two mapping populations (MP-EDITS1 and MP-EDITS2, generated by JIRCAS) to ensure the sustainable use of plant materials for yam breeding programs in Africa. For the 1<sup>st</sup> batch of the non-redundant set, all materials of the 119 lines were shared with the IITA yam breeding unit in tuber form (with full phenotyping data set), and several materials have been nominated and used as parental materials of crossing activities in Ibadan HQ and the Abuja station. In addition to tuber materials, currently, 74 of 119 lines (62%) were maintained in in-vitro form (meristem culture), and the attempt to generate meristem cultures from the remaining materials to complete 119 lines is ongoing with the revised tissue culture manual at the IITA Bioscience Center. These materials had wide genetic diversity in both agronomic and tuber quality traits. The re-sequence data set generated by IBRC is also available and is an important standard reference material for use in various activities. To generate virus-free materials to enable further material sharing with the partners in other countries, the available in-vitro plantlets managed by JIRCAS were transferred to the IITA Genetic Resources Center (GRC). In Nov 2018, six lines were confirmed virus free, ready for international sharing, and currently planned to ship to Japan in April 2019. The attempt to generate more virus-free materials of non-redundant sets is ongoing at IITA GRC. The two mapping populations, MP-EDITS1 and MP-EDITS 2, have also been maintained in both tuber form (249 and 208 lines, respectively, in 2018) and as in-vitro plantlets (meristem culture, 244 and 215 lines, respectively, in 2018) and are ready for sharing with partners in Nigeria. How and where these materials (especially MP-EDITS2 with re-sequencing data generated by IBRC) should be sustainably managed have been actively discussed among the IITA Yam Breeding Unit, IITA GRC, and JIRCAS. There is need for urgent decision on this issue. An institutional arrangement should be in place to prevent the loss of these valuable germplasm.

#### Whole genome re-sequencing of 300 *D. rotundata* genotypes for genome-wide association study (GWAS)

IBRC completed the resequencing of 333 accessions of Guinea yam and provided all the data to IITA. Sequence depth of each accession was more than  $\times 10$ . Using the sequence data, a total of 1,623,483 SNPs were extracted for further analysis.

In IITA, the level of genetic differentiation in the white yam diversity panel was dissected with BIC and discriminant analysis. The result of the discriminant analysis grouped the 333 yam clones into four major clusters with varying numbers of clones assigned to the different groups. Based on the Identity by State (IBS) matrix, a genetic distance of  $GD \leq 0.04$  was defined as a threshold for duplicates. Using this threshold, out of the total 332 clones analyzed, 188 clones were found to be unique. The unique clones identified herewith along with grouping based on genetic distance are highly relevant for the white yam breeding program to develop heterotic groups and exploit their unique features through recurrent selection. From the three major group of germplasm analyzed (breeding line, farmers' varieties, and Genebank accessions), a high number of duplicates was observed in the Genebank accessions follow by the breeding lines. The high rate of duplicate clones in Genebank accessions might be due to the collection of genetically identical accessions with different identities at different collection sites. Through the phylogeny analysis, duplicate clones among the Genebank collections, breeding lines, and farmer varieties were also identified. Clones in the same genetic branch are considered as duplicates. Most interestingly, the most preferred and popular farmer varieties in Nigeria namely Hembakoase, Akwuchi, Meccakusa, and Ojuyawo were found to be the same clones but known with different names. Similarly, the breeding line TDr8902157 has corresponding duplicate genebank accessions TDr2038, TDr2630, TDr3430, TDr3294,

**Commented [JL13]:** What decision was taken?

**Response:** Decision has not yet been taken on how to conserve the materials. We plan to meet with JIRCAS and IITA GRU to resolve the issue.

**Commented [JL14]:** Presumably this was a surprise? What was the ratio in the original Edits group that had been genotyped by SSR? Was the second diversity set also genotyped by SSR?

**Response:** 100 clones were genotyped using the SSR marker. With my knowledge, the second set wasn't genotyped by SSR.

**Commented [JL15]:** Maybe, genetic distance is not always a good proxy for heterotic grouping, but can be a useful starting point.

**Response:** This is noted.



TDr8902475, TDr608, and TDr2080. The breeding line TDr9600629 was also found to be a corresponding duplicate of TDr1707, TDr1707A, TDr2349, and TDr3647. The breeding lines TDr9601818 and TDr8700211 were found to be duplicate clones. The diversity panel was created using the passport and GBS data analysis, but we realized that it was not very efficient in identifying the unique clones. With very informative SNP markers from the whole genome resequencing, it is now possible to retain unique clones for further genetic analysis and breeding by the yam program. Details of the results obtained are showed in Appendix 1.

IITA also performed a preliminary association analysis. Two key traits were selected from the 2017 phenotyping for this preliminary genome wide association analysis. The traits were Yam Mosaic Virus Disease resistance (YMVD) and plant sex. Association analysis was performed using the unique clones after dropping the potential duplicates based on the IBS and genetic distance. From the 333 clones genotyped, 188 were identified as unique in the panel. From the 188 unique clones, 178 with the phenotypic data were considered for this preliminary association analysis. A Diploid HapMap file was created and subjected to the mixed linear model analysis using population structure and kinship as cofactors in TASSEL software. To add kinship as cofactor in the model, principal component analysis using the kinship matrix was performed. The first 10 PCs (PCs = 10) were used as a cofactor in the frequentist association test model in TASSEL. The P value and the molecular marker effect were estimated using GAPIT. The effective number of independent 70793 SNPs was calculated using the LD pruning. A marker with high LOD was considered as significant and associated with the considered traits. QQ plot alongside the Manhattan plot were then plotted using qqman and CMplot (both are R packages). YMVD: four independent associations distributed on four chromosomes were identified. The Manhattan plot revealed the presence of high LOD at chromosome 3 with a LOG > 7 (Details in Appendix 1).

Similar analysis for plant sex identified eight associations distributed across different chromosomes. Out of the eight hot spots identified with this analysis, none was found on chromosome 11 which was previously reported as a female-specific putative genome region controlling flower sex in white yam (Tamiru et al. 2017). This might indicate the presence of other potential regions of genome modulating flower sex expression of white yam in addition to the already reported region in pseudo-chromosome 11. Robust analysis methodology will be implemented using data from two seasons to successfully report genetic variants influencing the expression of key traits in white yam.

#### Mapping populations Genotyped By Sequencing (GBS) to generate high-density SNP marker data and identification of important QTLs and genes by whole genome re-sequencing (WGRS) of bulked DNAs

In IITA both *D. alata* and *D. rotundata* bi-parental mapping populations were tissue sampled and sent for genotyping at Toulouse, France. All progenies from the 2017 season planting and some newly added progenies from the 2018 season planting were sampled for genotyping. The *D. alata* bi-mapping population (TDa1401) was sequenced in two batches. The first batch consisted of 220 progenies from 2017 planting and the second batch included the newly added 74 clones from 2018 phenotyping to augment those progenies that failed to produce tubers in 2017 and were lost in the process. Two to three young leaves were collected from full-grown plant and the leaves were lyophilized before sending to CIRAD, Montpellier, France for sequencing. DNA was extracted using the full method implemented by CIRAD for yam using a Qiagen kit. DNA quality and concentrations were assessed using lambda DNA and agarose gel. SNP data were generated using GBS platform and illumina high sequencing density and SNP was called in collaboration with CIRAD using an open source web site. GBSX, a java script based toolkit, was used for the mapping (<https://github.com/GenomicsCoreLeuven/GBSX>) while for the demultiplexing, cutadapt (<https://cutadapt.readthedocs.io/en/stable/#>) was used and for the treating reads, vcfhunter/process\_reseq (<https://github.com/SouthGreenPlatform/VcfHunter>) was used. In general, the raw reads were mapped with the yam genome reference (<https://www.ncbi.nlm.nih.gov/genome/?term=Dioscorea+rotundata>). For *D. alata* (TDa1401) the SNP call was done with the first batch of 220 progenies and 16 292 SNP were identified. These have not yet been pruned to retain the final SNPs for subsequent analysis.

**Commented [JL16]:** Were the breeding number designations likely accurate, or were they mislabeled somewhere and actually represent lines collected in farmers' fields?

**Response:** The GRC usually recode collected materials (breeding lines and farmer's varieties) so there is a possibility of duplication as a result of the recoding. GRC collected material from year to year and tendency to collect same material is very high.

**Commented [JL17]:** Who analyzed the GBS data?

**Response:** The GBS data was analyzed by Gisel and David in IITA. The material for the GWAS was selected and sent to IBRC for Sequencing. Agre then reanalyzed the data and duplicates were found but the GBS quality was low.

**Commented [JL18]:** Did you compare data for putative "duplicates" to ensure that they had very similar scores? If not, which one was the "right" one to use?

**Response:** Yes through different methods. The right one to use was chosen based on the seed availability and the phenotype data over the two years. However none of the clones is discarded as special observation will still be carried out on the duplicate clone

**Commented [JL19]:** Definition of "high LOD"?

**Response:** Above the suggestive LOD value

**Commented [JL20]:** How do you explain this? Are there multiple controlling loci?

**Response:** Yes, there could be a possibility of multiple loci controlling plant sex information in yam.

**Commented [JL21]:** Which methodology that is known to be "robust analysis methodology"?

**Response:** Developing different format such as additive, additive dominant and additive recessive and using different 'r' packages and tools for the GWAS

With *D. rotundata* (TDr1402) mapping population, substantial progress was made with SNP calling, pruning, and map construction. A total of 6,847,845 SNPs was obtained after read mapping. The raw SNPs were pruned with high quality control criteria: Read depth > 5, Genotype quality > 20, no indels, minor alleles frequency > 0.05, no missing, minimum and maximum alleles = 20. Makers within the same LD were removed through windows-size LD before the data was pruned. A total of 6732 SNP was retained for genetic map construction and for QTL detection. For the analysis and using the yam genome reference, VCF was modified as the chromosome names weren't mapped with the algorithm to be used for the imputation. SNP distribution was assessed by splitting the VCF files based on the chromosome number. The number of SNPs per chromosome varies from 142 to 739. The highest number of SNPs was observed on chromosome 5 (n = 739) followed by chromosome 8 (n = 622), while the lowest SNP number was obtained on chromosome 21 (n = 142). More details of the results obtained are in Appendix 1.

The development of a robust analysis pipeline for dissecting marker trait association with bi-parental mapping population and diversity panels is still in process at IITA. All populations were genotyped, and high-density SNPs were identified or in the processes of variant calling. The discovery of informative markers associated with key traits is yet to be done. In the meantime, attempt was made to identify informative SNPs from GBS and whole genome resequencing data and use them for routine quality assurance and control in yam breeding programs. As part of this effort, 46 SNPs were converted to KASPAR assay with LGC chemistry. KASPAR-converted SNP markers were validated on 273 diverse clones and over 1300 breeding lines. The validation data on 273 clones was uploaded to the YamBase and data can be viewed using this link: <https://yambase.org/breeders/trial/444?format>. Furthermore, genotypic data generated for the breeding progenies were also uploaded in YamBase and are available through this link: <https://yambase.org/breeders/trial/445?format>.

IBRC focused on F1 family MP2 maintained in IITA. MP2 was derived from a cross between landraces TDr04-219 (P1: female parent) and TDr97/00777 (P2: male parent). All F1 progeny have been sequenced using illumina platform with average sequence depth being more than  $\times 10$ . After alignment of the short reads to the reference genome (TDr96\_F1), SNP information was extracted. Two types of SNPs (Types A and B) were used for association study. Type A SNPs (256,435 SNPs) were heterozygous in female parent (P1) but homozygous in male parent (P2). Type B SNPs (280,733) were heterozygous in P2 but homozygous in P1. Phenotype data of MP2 family have been provided by IITA and JIRCAS. Genome-wide association study (GWAS) using these Type A SNPs showed that tuber flesh color showed significant associations with four genomic regions (qTFC1, qTFC4, qTFC12, and qTFC14). The region on pseudo-chromosome 4 (qTFC4) showed the highest level of association ( $-\log_{10}P > 6$ ). The genomic region of qTFC4 contained 77 genes. One of them, Dr10160, encoded cytochrome P450 protein with a similarity to fusca protein known to be involved in anthocyanin accumulation. RNA-seq study showed that Dr10160 is expressed in tuber. It is therefore hypothesized that Dr10160 is a candidate gene responsible for the difference of tuber flesh color in MP2. IBRC also already developed DNA markers for diagnosis of plant sex (Tamiru et al. 2017). These are expected to contribute to the diagnosis of sex of plants to be used for crossing. DNA markers are also being developed for the QTL controlling stem number per plant. Once validated, it will be used in other segregating populations to see its transferability and eventually used as a DNA marker associated with stem number. Plant materials are needed from different families to test these markers. Using the SNP information, IBRC carried out GWAS for some traits including tuber number, total tuber yield, stem number, sex, days to flowering, days to start of senescence. Other analysis carried out include preliminary population genomics analysis to understand genetic diversity of Guinea yams, determination of phylogenetic relationship of the 333 accessions based on SNP data, and the detection of selective sweep by genomic scan. Details of the results are of these analyses are in Appendix 1.

At CIRAD, genotyping of the progenies of the two bi-parental mapping populations of *D. alata* was performed by GBS (genotyping by sequencing) to detect large numbers of SNP codominant markers. Both populations were

**Commented [JL22]:** What was the process used to identify these 46 SNPs as worth developing KASP markers for?

**Response:** 192 SNPs marker were selected from the previous GBS data (maf>0.1, no missing, high pic, SNP pruning was performed using window size the parameters for --indep are: window size in SNPs (10) with R square = 0.1.

**Commented [JL23]:** In other species, a third type of SNPs is also used in such analyses: SNPs that are heterozygous in both parents (P1 & P2) and segregate 3:1. These form useful bridges to independently connect the two parental maps.

**Response by IBRC:** SNPs that are heterozygous in both parents will be included and the QTL analysis for all segregating traits among F1 will be re-done. IBRC is also currently generating Guinea yam reference genome version.2 and all QTL analysis will be carried out again with this new reference. They hope to report the new results as soon as possible.

**Commented [JL24]:** If the dominant color being determined is yellow, would the biochemistry be expected to be related to anthocyanin accumulation? I would have thought you might be looking for something affecting carotenoids (P450 genes also involved in those pathways). Or are you detecting reds, blues, etc. that would be associated with anthocyanins?

**Response by IBRC:** Yes, tuber flesh color of F1 is between yellow and white, so that carotenoid is more likely to be involved in the color difference. We will look for other genes in the region that may be involved in carotenoid biosynthesis and provide you with the updates in the next report

generated at the beginning of the project by hand pollinations between contrasted diploid genitors. Population A (74F x Kabusa) is composed of 121 progenies while population B (74F x 14M) has 193 progenies.

Using the same female and the same type of markers, it was possible to generate a high-density consensus genetic map that will be useful to identify possible QTL clusters and to check if the QTLs detected in one progeny for a given trait correspond to QTLs identified in the other population for the same or a related trait. The construction of consensus genetic maps was performed using JoinMap 4.1. Linkage analysis and map constructions were performed for each parent by family using JoinMap 4.1 software from the GBS data generated in 2017. Regression algorithm was used instead of the maximum likelihood mapping algorithm MML, because the latter provided an increment of map length. Linkage groups were established using a grouping LOD threshold value of seven for population A (74F x Kabusa) and four for population B (74F x 14M). Parental maps were computed using a recombination frequency below 0.450, LOD superior to 1, and the Kosambi mapping function.

The consensus map was generated using the recombination frequencies. This approach is more restrictive than the construction of consensus map from maps projections. However, it allows a more robust estimation of SNP order. As the two mapping populations were derived from crosses involving the same female (74F), an integrated map of the female was first computed using the “combine groups for map integration” function. Then, the final consensus map was constructed using this function and starting from the consensus female group and both male parent groups. Visualization of homology between the four parental maps, the integrated female map, and the final consensus map were performed using R cran ggplot2 library. The consensus map obtained was 2547.3 cM and contained a total of 1548 SNPs distributed on 20 linkage groups (Details shown in appendix 1). The average marker density was 1.67 cM/marker. Linkage groups were numbered in reference to the *D. rotundata* genome (Tamiru et al. 2017). Linkage groups contained from 145 (LG 5) to 20 SNPs (LG14) and a genetic length from 188cM (LG 5) to 55.6 cM (LG14).

A QTL analysis was conducted (in two bi- parental mapping populations of *D. alata*) on data collected in 2018 to acquire knowledge about the genetic control of different characters that determine the tuber quality and to identify genomic regions involved in quality traits. The analysis was conducted by the interval mapping procedure using the MapQTL 6.0 package. Permutation tests (1000 permutations) were performed to obtain the significance LOD threshold. The result showed that the observed distribution of tuber shape (ratio length/width) for individuals of populations A and B deviated from normality and were close to bimodal.

Several significant QTLs were detected on both populations (74F x Kabusa and 74F x 14M). For population B (74F x 14M) the tuber shape (ratio length/width) is the character with the highest LOD value (16.53) and the one with the highest coefficient of variation (93.99%). This QTL explained 54.4% of the phenotypic variance and is associated with the marker 16.1\_20695335 which is on linkage group LG16. A QTL was identified on population A on the same linkage group LG16 that explained 37% of the phenotypic variance. A QTL was also identified on population A with effects on the regularity of tuber shape on linkage group LG15, which explained 25.2% of the phenotypic variance. This QTL is associated with the marker 15.1\_8782114 which is of a maternal origin (type Im x Il). A QTL was identified on population B with effects on the regularity of tuber shape on the linkage group (LG16), which explained 20.2% of the phenotypic variance. This QTL is associated with marker 16.1\_21690061. Similarly, a QTL with effect on hairiness was identified on population B on linkage group LG15 which explained 17.7% of the phenotypic variance. Detailed results are shown in Appendix 1.

#### **Phenotype non-redundant set for tuber quality, YMV, anthracnose, nematode resistance, tuber dormancy, earliness, tuber morphology and starch properties**

The GWAS panel of *D. rotundata* and *D. alata* were successfully phenotyped for different agronomic, disease, and quality traits. The *D. rotundata* panel was field phenotyped for two consecutive seasons (2017 and 2018) at one location while the *D. alata* panel was phenotyped for one year at three locations.

***D. rotundata* GWAS panel:** Phenotyping of the *D. rotundata* panel was started in 2017 using 319 yam accessions composed of 113 breeding lines, 193 genebank accessions, and 13 farmer varieties. The materials were

**Commented [JL25]:** of which parent?

**Response by CIRAD:** This marker is of a paternal origin (type nn x np)

**Commented [JL26]:** This was an independent QTL on the same chromosome?

**Response by CIRAD:** It's the same QTL detected on both populations on the same genomic region

**Commented [JL27]:** How is this defined?

**Response by CIRAD:** The measure of the regularity of tuber shape takes into account the level of deformity of the tuber (scored 0=absence of deformity, 1=low, 2=high deformity)

phenotyped for different traits using a partial replicated experimental design laid out in a single row plot of three plants in 1 m × 1 m inter and intra-row spacing. Forty-three accessions were replicated in four blocks while 276 accessions were planted as non-replicates. Vegetative and yield data were collected on 311 accessions that survived till harvest. In the 2018 cropping season, the GWAS trial was pre-sprouted in the screen house on 8 May 2018 and transplanted to the field starting from 1 June 2018. Of the 311 accessions harvested from the 2017 trial and pre-sprouted, 278 accessions were successfully established in the 2018 trial due to the loss of a few tubers in storage and non-sprouting of some others during the pre-sprouting exercise. The pre-sprouting was implemented both in the 2017 and 2018 seasons to establish uniform plant stands, thus ensuring quality data from the trials. The accessions were planted in three replications of a single plant each at a spacing of 1 m × 1 m in 2018. Data collected in both seasons included days to sprouting, days to flowering, days to maturity, number of stems, stem diameter, spines on stem, plant vigor, canopy architecture, leaf density, leaf shape, stem color, anthracnose and yam mosaic virus disease incidence and severity (starting two months after planting till six months at two-week interval), senescence class, plant sex, flowering intensity, inflorescence type, number of plants harvested, number of tubers, tuber length and width, tuber weight, appearance of tuber surface, tuber hairiness, spines on tuber surface and tuber dry matter content, tuber oxidation intensity, and starch property for 2018. (Tuber yield and quality traits data for the 2018 season have not yet been finalized to include in this report.) The field phenotyping work was a joint effort with JIRCAS while the whole-genome re-sequencing of the panel was done at IBRC, Japan. Phenotypic data collection was performed using the standard operating protocol for yam variety performance evaluation trial (Asfaw 2016) and the yam trait ontology on YamBase (<https://yambase.org/>).

**Preliminary result of *D. rotundata* panel:** Variations for phenotypic traits were assessed for the 2017 planting, 2018 planting, and the combined data for two years. The level of variation and statistical significance for the traits assessed in the 2017 season are presented in Appendix 1. Plant vigor, number of stems and stem diameter for the population ranged from 1.0, 1.0, and 1.7 cm to 3.0, 6.0, and 8.0 cm, respectively. Out of the 311 accessions that were usefully established in the field, 86 (27.7%) did not flower, 180 (57.9%) flowered male, 41 (13.2%) flowered female, while three (0.96%) were monoicous (male dominant). Days to flowering ranged from 30 to 115 days while the score for flowering intensity ranged from 3.0 (low flowering) to 7.0 (profuse flowering). Area under disease progression curve for YMV (AUDPC-YMV) and anthracnose (AUDPC-YAD) ranged from 259.1 and 174.3 to 521.9 and 390.5, respectively. Number of tubers per plant ranged from 1 to 5 while fresh tuber yield ranged from 0.4 t/ha to 27.9 t/ha. Means of some selected traits for the top 30 highest yielding accessions are presented in appendix 1. Fresh tuber yield for the top 30 accessions ranged from 16.28 to 27.85 t/ha.

Subjecting the phenotypic variables to principal component analysis identified the first five principal components explaining 52.2% of the variation encountered in the association panel. The first principal component accounted for 21.3% of the variation and illustrated primarily the variations in plant vigor, leaf density, tuber width, tuber length, days to start of senescence, fresh tuber yield, and average tuber weight. The second principal component accounted for an additional 10.2% of the total variation and described predominantly the patterns of variation in stem diameter and tuber area. The third principal component was responsible for an additional 8% of the variation and showed mainly the variations in senescence class, tuber appearance, and spines on stem. Similar assessment for the phenotypic variable from the 2018 field phenotyping showed significant difference for all the traits assessed in the association panel except for number of stems per plant and canopy architecture. Summary of the means score of some selected traits of the top 30 accessions based on their AUDPC-YMV are presented in Appendix 1.

***Dioscorea alata* diversity panel:** In all, 100 clones of *Dioscorea alata* were phenotyped at three different locations: Ibadan, Ubiaja, and Ikenne during the 2018 planting season. The field trials were laid out in a lattice design replicated three times using a single row plot of five plants arranged in an inter and intra row spacing of 1 m × 0.5 m. A total of 25 different variables were recorded using agreed yam crop ontology ([www.yambase.org](http://www.yambase.org)). The trial from Ibadan is under quality phenotyping including RVA, starch content, tuber flesh oxidation, and tuber dry matter. The phenotypic data collected were subject to various analysis pipelines. Preliminary results showed significant effect of genotype, location and genotype × location interaction for variation in expression of the different traits. The mean yam anthracnose disease (YAD) scores for Ibadan, Ikenne, and Ubiaja were 2.6, 2.5,

Commented [JL28]: Most traits weren't correlated at all?



and 2.34, respectively. The mean YAD severity score ranged from 2.19 to 3.36 at Ibadan, 2.28 to 3.02 at Ikenne, and 1.42 to 2.95 at Ubiaja. From these results it is evident that anthracnose disease pressure under field natural infestation was not too high. We also did detach leaf assay on the trial plot from Ibadan and data is under processing. Based on the YAD score, the top 10 best performing genotypes were selected as shown in Appendix 1. Among the 10 best clones identified across the three locations, TDa100029 at Ibadan and Ikenne, and TDa0200061 and TDa1100202 at Ikenne and Ubiaja had consistently low scored values for YAD severity, hence might be good genotypes conferring putative tolerance to YAD. Through the analysis of the boxplot, clones evaluated in Ubiaja seem to have low YAD scores across all the three replications while clones planted in Ikenne and Ibadan seem to be more exposed or susceptible to anthracnose disease.

Tubers of elite breeding lines from 2018 multi-location trials (both *D. alata* and *D. rotundata*) were supplied to the IITA nutrition laboratory for quality analysis. In addition, tubers from *D. alata* diversity panels and regional variety trials were under quality determination for RVA properties, tuber flesh color reading, tuber flesh oxidation, total starch content, and amylose content in collaboration with the JIRCAS team. We also implemented pounding and boiling quality determination of all advanced clone performance trials, regional variety trials, and parental clones in crossing block. The data is being processed and will be reported next year.

#### High through-put phenotyping technologies for food quality, anthracnose, YMV, and nematodes

In 2018, the disease phenotyping activities focused on the validation and use of the newly developed screening protocols. The Detached Leaf Assay (DLA) was used for YAD and phenotyping of *D. alata* populations (TDa 1401 mapping population). The “ESTIMATE” app was developed using YAD standard area diagrams (SAD) for image-based phenotyping in the field and DLA. This tool offers a series of images of diseased leaves with increasing symptom severity and values of percent symptomatic area for users to compare the symptoms on the test leaves and match with it with SADs to estimate the disease severity. The app relays data directly to user account for ready downloading for analysis. This app reduces time for assessment and improves accuracy of phenotyping. The tool is validated and will be disseminated to partners in 2019.

A method for mechanical inoculation of YMV was also perfected offering 95% transmission rate. This approach uses mechanical transmission of YMV from source plant to *N. benthamiana* and from *N. benthamiana* to yam seedlings and young plants (3- to 4-leaf stage) generated from tissue culture or SAH. This method has opened new opportunities for accurate YMV phenotyping of *D. rotundata* breeding populations. Semi-quantitative analysis of YMV has been standardized and will also be made available for use by our partners. SOP/protocols for the YAD screening has been uploaded on the AfricaYam web page.

#### YamBase

During the reporting period, BTI expanded content and functionality of the YamBase website. Building on the successes of the outreach to breeders and breeding programs, more features and tools were being added to the YamBase based on the feedback received from breeders and users of the database. The weekly breeders Skype meetings effectively supported the breeding programs in their use of YamBase. These have provided technical assistance to the breeding programs and more feedback on adding new tools and functionalities as well as optimizing already implemented tools and features in the database. Several tools were added to the database and website during the period under review. These include, among others, improvement of field layout visualization tool and assayed trait visualization heatmap; more printing options added to the interactive Label Designer to capture new datatypes added to the database; workflows added to guide the use of complex tools; and the general YamBase usage. The database now counts over 54 000 accessions with over 1500 genotypes, 7 breeding programs, 197 assayed traits, about 414,000 phenotype scores, and over 325 trials. Website functionality has been adapted to breeder's input. The database has contributed to harmonizing data and standardizing procedures across breeding programs. It has also enhanced collaboration across partners and supporting YCoP.

**Commented [JL29]:** You have 4 numbers for 3 locations here, including one that is out-of-range (7).

**Response:** This is an error. 7 is deleted.

**Commented [JL30]:** Are the scores meaningful under low disease pressure?

**Commented [JL31]:** Is it reasonable to discuss comparative YAD performance when you have not had a good field challenge?

**Response:** Yes it is not reasonable to compare but the main observation was some clones that showed susceptible reaction in the field turned tolerance under detached leaf assay and vice versa.

**Commented [JL32]:** Using what quality parameters at the IITA nutrition lab? What specific tests are being done?

**Commented [JL33]:** What is the relevance of this assay if not validated by field screening? How do you decide which isolate is most appropriate to use?

**Response:** YAD incidence and severity under field conditions depends on the three way interaction between host, pathogen strain and environmental conditions and generally subjected to high G x E bias. Inoculation under controlled conditions reduces G x E bias, and offer best estimate of host response against a specific Cg isolate. We have been using Cg KGO1, isolated from Kogi state in Nigeria, which was found to be highly virulent. It is possible to test a genotype against a broad spectrum of Cg isolates with ease using DLA.

Previously we compared field, screenhouse (whole plant assay under controlled conditions) and DLA methods which demonstrated high correlation between screenhouse and DLA (see Nwadieli et al. 2017; <http://dx.doi.org/10.1094/PDIS-06-16-0924-RE>; copy attached).

Reliable assessment under field conditions requires testing in a 'hot spot' which offers conducive environmental conditions and creation of a sick field. DLA offers convenient alternative for preliminary screening and further testing can be done in multilocations.

**Commented [JL34]:** How does this method compare to multi-year field screening results for the same genotypes?

**Response:** In 2018, only two genotypes were used for testing by mechanical inoculation, and the data correlated with field infection by aphids (virus-free seed planted) and tuber-borne infection (virus-infected seed planted). We could not test more accession due to limitation of virus-free plants. We will perform more tests this year.



### PRIMARY OUTCOME 3

#### **In-country testing of *D. rotundata* and *D. alata* elite clones**

In-country multi-location testing (MLT) of *D. rotundata* and *D. alata* elite clones was carried out in Nigeria for two consecutive years (2016 and 2017). The best performing clones for agronomic and quality traits were advanced to on-farm variety verification trials in 2018. The variety verification trial consists of three candidate clones, one standard check, and one local check for both *D. rotundata* and *D. alata*. The verification plots were planted in more than 15 on-farm plots in the 2018 season in collaboration with NRCRI, Umudike. The on-farm plots were visited by national variety release technical committee members at the vegetative stage. This inspection visit was sequel to a letter of invitation to the Registrar, National Crop Varieties and Livestock Breeds Registration and Release Committee on 21 of September 2018. The committee representatives consists of two independent teams. The first team assigned to cover the South-east and North-central comprised Dr Kenneth Omokhaye, Member Technical Sub-Committee; National Varietal Release Committee (Director, Biotechnology Department, Rubber Research Institute of Nigeria (RRIN), Benin, Edo State); and Dr Anthony U. Okere, Secretariat, National Varietal Release Committee (Asst. Director Research and Development, National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State); while the second team for South-south and Southwest region comprised Prof. S.A. Olakajo, National Co-Coordinator Maize, Institute of Agriculture Research and Training (IAR&T), Ibadan, Oyo State and Mr. Clement Michael, Secretariat, National Varietal Release Committee (Research Scientist), National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State). The activities of the respective teams were to ascertain the desirability of the nominated candidate varieties and the suitability of the on-farm trial locations as well as their management. To this end, the farmers were interviewed while vegetative criteria ranking of the candidate varieties were carried out in most of the locations. Subsequently, recommendations on the remaining process for the release of best candidates were given by the team. We also executed participatory selection and consumer acceptability assessment for boiled and pounded products from the on-farm farmer-managed verification plots. This exercise was done by two separate teams: one from IITA on verifications planted at Oyo North and Edo states and the other by NRCRI team on verification planted at South East states. The activities carried out during PVS were tuber evaluation, ranking selection criteria, ranking clones for tuber traits and yam products (boiled and pounded yams of the candidate and check varieties from verification plot). In each on-farm location, 20 experienced or knowledgeable farmers/panelists on yam production and consumption from the surrounding communities were registered for criteria ranking and tuber assessment/ranking, precisely 10 men and 10 women. Group discussion was had with the selected farmers to list the best criteria farmers use to assess or describe yam genotypes at harvest (visible tuber) and post-harvest (cooked and pounded yam). The names of each trait/criterion were written boldly on a paper bag spread out wide. Each panelist/farmer was given six grains of maize (men) and six grains of beans (women) for voting. The instruction was to drop three grains in the bag bearing their best criteria, two grains in the 2<sup>nd</sup> best, and 1 grain in the 3<sup>rd</sup> best for describing yam varieties or products. The panelists/farmers were to do this independently without influence from any other member of the panel. Counting was openly done at the end of the voting to announce which trait/criteria took the lead and the rank was announced. Each harvested variety was labeled with code number plot 1 to 5 boldly, a paper bag was placed openly in front of arranged tubers. Panelists were provided with six maize seeds for men and six bean seeds for women, after which voting was done in order of three seeds for 1<sup>st</sup> chosen genotype, two seeds for 2<sup>nd</sup> best, and one seed for the 3<sup>rd</sup> best variety harvest from the verification plot (this exercise was independently done for the *D. rotundata* and *D. alata* clones). This was done independently without influence from other panelists, followed by counting, and then the winner clones are announced. Names (local names) were unanimously given to the selected clones by farmers in each location. The RTBFoods sensory assessment was carried out in two locations Kisi and Atani, using six processors and two assistants. General evaluation was done at tuber after harvest, at peeling, boiling, and pounding. For boiled yam product assessment, the following traits were considered by farmers: texture, aroma, color, taste, and overall quality of the product. The assessment was done using a 1–5 scale for all traits: 1 = like extremely, 2 = like, 3 = neither like nor dislike, 4 = dislike, and 5 = dislike extremely. For texture a 1–3 scale was used: 1 = strong, 2 = intermediate, and 3 = soft. Overall quality was assessed using 1 = very good, 2 = good, 3 = fair, 4 = bad, 5 = very bad. The data on consumer acceptability and sensory assessment for yam products is under process.

UAC held a meeting with key members of the plant variety release committee of Benin. Four members of the Catalog Committee (the variety release committee in Benin) visited the trials at Massi, Dassa, and Tallou and in three farmers' fields to see the performance of the varieties to be released. They recommended the submission of the list of the varieties to be released after statistical analysis. The variety development procedure was discussed and the list of the varieties to be released was finalized. Five varieties were also submitted for release at CNRA. The process could not be concluded during the reporting period because the release committee was not active.

#### **Regional trials of *D. alata* and *D. rotundata***

The regional variety trials (RVTs) were established in 2017 and continued in each country in 2018. The trials composed of 16 clones each for the *D. alata* and *D. rotundata*. Of the 16 clones, 14 were test clones while one was the common standard check across sites and one local check specific to the site. The RVTs were arranged in a triple lattice design and planted at three sites in Nigeria, two sites in Ghana, and one site each in Benin and Cote d'Ivoire in the 2017 cropping season. In the 2018 cropping season, the RVTs were repeated at eight sites in Nigeria, four sites in Ghana, two in Benin, and one in Cote d'Ivoire with minor modifications: partners in Cote d'Ivoire selected promising clones from first season planting while in Benin some clones were added to increase the number to 20. Data collection and analysis by partners is in progress. The milestone for this activity was fully met.

#### **Development of *D. rotundata* and *D. alata* populations with multiple priority traits including yam mosaic virus and anthracnose resistance**

The yam-breeding program primarily targets agronomic traits (tuber yield attributes), biotic stress resistance traits (virus tolerance or recovery in *D. rotundata* and anthracnose resistance in *D. alata* breeding pipelines), and quality traits (tuber flesh enzymatic oxidation or browning after peeling or processing and tuber dry matter). The program also covers a range of other traits that describe the performance and acceptability of new varieties in production and consumption systems. In the 2018 cropping season, the yam breeding activities covered the entire continuum of breeding stages that span across making crosses to generate new variability, management of seedling and tuber progenies, and early generation clonal selection and evaluation trials to orientate the variability to the defined target, identify new series of advanced clones for validation of their superiority in production and consumption systems, and choose new clones with parental value for further recombination generating better population for future selection. New crosses were made in 2018 to generate new series of *D. rotundata* populations at IITA. Ten female and five male parents identified based on their good breeding value prediction were hand pollinated at Abuja. In addition, controlled hand pollinations were made in *D. rotundata* advanced performance evaluation plot to generating a second cycle recurrent selection population. Five females and two males were open pollinated in a polycross isolation nursery at Ago-Owu. The newly created breeding populations from 2017 crosses were assessed under seedling nursery in 2018 while some botanical seeds of *D. rotundata* and *D. alata* populations from 2017 crosses that combined putative virus and anthracnose resistance with other priority traits were successfully shared with partners (CSIR-SARI, NRCRI, EBSU), and partners successfully generated seedlings in 2018. About 2,800 seeds were sent to NRCRI, 5,050 to SARI, Ghana, and also to EBSU, Nigeria. The seedling progeny nurseries generated at IITA encompass both full and half-sib progenies involving multiple parents. The seedling progenies managed in 2018 were 23 and 40 half-sib families of *D. rotundata* at Ibadan and Abuja, respectively, and 37 and 34 full-sib families of *D. rotundata* and *D. alata*, respectively, at Abuja. In general, 2174 full-sib and 614 half-sib progenies of *D. rotundata* and 778 full-sib *D. alata* progenies were generated in 2018 both under screen house and direct transplant in the field. These materials were harvested and selected for qualitative traits such as tuber appearance and smoothness, tuber shape and size, and were advanced to tuber family evaluation trials in 2019 for further assessment and single plant selection to develop a new series of clones. In addition to the seedling progenies, 51 full-sib tuber families of *D. alata*, 27 full-sib and 65 half-sib tuber families of *D. rotundata* were evaluated at Abuja. At Ibadan, 40 half-sib tuber families of *D. rotundata* were evaluated. Family evaluation and single plant selection were conducted to reconstitute new series of clones (clonal selection).

The selected single plants will be advanced to tuber-to-row plots to generate second clonal generation (SCG) nurseries in the 2019 cropping season. Over 400 second clonal generation (SGC) *D. rotundata* clones were raised in an augmented block design for selection and breeding value prediction of the parental clones based on progeny performance. Harvesting and phenotypic selection on SCG were completed and this will be supported with detailed analysis to apply index selection to identify promising clones to be advanced to the next breeding stage. Dry matter content of 25%, tuber yield advantage of 5% over check, virus severity score of less than 2.5, and no tuber flesh oxidation for at least 180 minutes after peeling or processing were set as cut-off points for advancing promising clones to the next breeding stage. Preliminary clonal performance evaluation trials of *D. rotundata* and *D. alata* each comprising 64 and 85 clones, respectively, were executed under lattice experimental design to identify superior clones for multi-location trials and those with good parental value for inclusion in crossing block to generate next cycle population development under recurrent selection strategy. The yam variety development pipelines operated from two hubs in Nigeria. The details of the different stages of the breeding trials executed in the 2018 cropping season according to the workplan is showed in Appendix 1.

#### **Breeding trials operated at Abuja station**

One crossing block and 11 different stages of breeding trials were planted in Abuja during the 2018 growing seasons in lattice, single replication, and augmented designs. The PPT, APTs, biomass partitioning, and drought trials were of lattice design, all second clonal generations were of augmented design, while crossing blocks and tuber families were of single replications. The spacing used for the trials were 1 m × 1 m with a general sprout count of about 80% and a total plot of 1245 with different plot sizes. The remaining planting materials were planted for multiplication at a spacing of 1 m × 0.25 m. The trials were planted beginning from 4 April all through to 14 May 2018. Some of the genotypes involved in the lattice design were randomly assigned to three replications based on the availability of tubers (planting materials) and each weight was taken before planting. Most of the genotypes from the trials produced tubers at harvest most especially TDr APT Set 2, which had big tubers though not of good shape which might be because of the hard soil structure as the tubers could not penetrate deep down into it. Data collected were number of emerged plants, stem number, plant vigor, anthracnose, yam mosaic virus (incidence and severity) for 2, 3, 4, 5, and 6 months, respectively; senescence class; days to flowering; plant sex and flowering intensity; as well as yield components including number of plants harvested, number of tubers, tuber length and width, tuber weight (categorized as big, medium, and small), hairiness, thorns, tuber surface crack, and tuber surface texture. All data were collected based on the standard operating protocol for yam variety performance evaluation trial (Asfaw 2016) and the yam trait ontology on YamBase (<https://yambase.org/>). Chroma Meter readings were also taken in the trials on yam tubers cut longitudinally and peeled, well packaged in Ziploc bags; the Chroma Meter was used to take readings three times on different portion at different time intervals of 0, 30, and 180 minutes, respectively, to monitor color change or oxidation. Dry matter was also recorded for all trials except single plant selection and seedling progeny nurseries. Dry matter content was determined by cutting the yams longitudinally, dicing, and weighing 150 g from each sampled genotype and placing in the oven for 48 hours at 100 °C. The dry content was weighed after oven drying and taken as a percentage of the initial weight which gave the exact dry matter percentage in the tuber for each genotype/clone.

**Seedling progeny nurseries:** The seedling nursery established at Abuja was composed of 37 full-sib families of *D. rotundata*, 34 full-sib families of *D. alata*, and 40 half-sibs of *D. rotundata*. The botanical seeds sown were 4836 *D. rotundata* FS, 2829 *D. alata* FS, and 2640 *D. rotundata* HS. The seeds were sown in the screen house beginning 5 April to 18 April 2018. Survived seeds were transplanted into bigger pots within the screen house with a survival count for TDr seedlings as 2174 while that of TDa seedlings was 778. The half-sibs were transplanted to the field to see its performance based on environmental factors; appropriate management (weeding, fertilizer application, trailing, wetting, etc.) was done from time to time. A total of 2196 seedling tubers were harvested from the TDr seedlings with a weight ranging from 1 to 228 g. From the *alata* seedling nursery raised in the screen house about 864 seedling tubers were harvested with weight ranging from 1 to 322 g while from

direct field transplanted HS seedling, 225 seedling tubers were harvested with weight ranging from 1 to 269 g. We generally noticed poor germination of the seeds and poor performance of direct transplant seedlings under field conditions due to poor soil and moisture conditions in the field.

**Tuber progeny nurseries:** Tubers from the 2017-seedling progeny nursery comprising *D. rotundata* and *D. alata* families were sorted out and planted as four tuber family nurseries namely TDrTuberfamily1, TDrHsTuberfamily1, TDrHsTuberfamily2, and TDaTuber family. TDrTuberfamily1 had 27 families and was established on the field in a replicated family plot on 8 May 2018, TDrHSTuberfamily1 had 32 families and established on the field in an unreplicated family plot on 8 May 2018, TDrHSTuberfamily2 had 48 families and established as a single replicate family plot on 16 May 2018, and TDaTuberfamily had 51 families and established on the field in replicated family plot. Data was collected on all trials on family basis for YMV and YAD responses. At harvest, data was recorded for the tuber yield component traits such as number and weight. Traits on tuber characteristics such as tuber appearance, cracks, shape, and thorniness were also collected. A total of 29 families were harvested from TDrHSTuberfamily1 with a total tuber number of 291 weighing from 4 g to 1455 g, 88 families were harvested from TDrHSTuberfamily2 with a total tuber number of 487 weighing from 4 g to 967 g, 34 families were harvested from TDrTuberfamily1 with a total tuber number of 1043 weighing from 16 g to 2476 g, and 30 families were harvested from TDaTuberfamily with a total tuber number of 294 weighing from 13 g to 1401 g per tuber.

**Second clonal generation nursery:** The second clonal generation assessed at Abuja was arranged in two sets: one as breeding population and the other set for genetic study. The genetic study set was assembled from four crosses consisting of two female and two male parents in a nested crossing arrangement. Both sets were arranged in an augmented trial design in a single row plot of three plants where checks and parents were replicated. The summary statistics of all the traits examined on second clonal generation nurseries at Abuja in 2018 are showed in Appendix 1. Phenotypic visual selection was attempted to promote promising clones to the next stage of the breeding trial. The visual selection was based on tuber characteristics (size, shape, appearance, tolerance to dry rot) using the standard check as benchmark for selection. The visual selection will be complemented with the detailed analysis for recorded data and construction of the selection index for key traits.

**Preliminary performance trial for yield and tuber quality:** This trial was advanced from the 2017 second clonal generation at Abuja to examine the performance for tuber yield and quality to select promising elite clones for multi-location trials. The trial was composed of 64 clones arranged in a triple lattice design with a single row plot of five plants. Traits recorded included emerged plants, severity and incidence for virus and anthracnose infestation, stem number per plant, and yield component trait. The preliminary analysis showed significant genotypic differences for some traits but not for some others. This analysis is very preliminary and has not considered the outlier and missing data. We will incorporate data quality assessment and also incorporate pedigree information to improve the analysis result and prediction of clonal performance. The selection index will be constructed to advance promising and superior clones to the next stage trial in 2019 compared to standard and local checks.

**Advanced performance trials:** The advanced performance trial was arranged in two sets: TDr APT Sets 1 & 2. Set-1 trial was composed of 16 clones replicated three times in a lattice trial design. Set-2 was constructed by combining superior selections from TDr CatA and TDr CatB that were advanced from separate trials at Ibadan and Abuja, respectively, in the 2017 calendar year. Set-2 was composed of 25 clones arranged in a triple lattice design. Both trials were a single row plot of 10 plants per replication. All preharvest traits and yield component traits were taken. Details of the results of the preliminary analysis for various traits assessed in the trials is presented in Appendix 1. Promising elite clones from the two sets will be advanced to multi-location trials in 2019.

**Efficiency of polycross and time of pollination on promoting fruit set in *D. rotundata* and *D. alata*** Efficiency of polycross on the generation of half-sibs was assessed on *D. rotundata* while the effect of pollination timing

was assessed on both *D. rotundata* and *D. alata* clones. Two sets of polycross nurseries composed of five females and one male each were established at Ago-Owu site to assess the efficiency of the site in promoting natural pollination and fruit set. However, there were natural pollination events, but the success was not very impressive as very few fruit and seed set were observed. We also assessed the effect of pollination timing on pollination success and fruit development on four clones (two *D. rotundata* and two *D. alata*). This experiment was to determine the appropriate time of pollen collection for hand pollination to support the crossing operation in yams. With this experiment, controlled hybridization was carried out at different hours of the day using viability tested pollens. Normal flower bagging was carried on all the plants before anthesis and the plants were hand pollinated with viability tested pollens at different hours of a day: morning hours (8 a.m to 10 a.m.), noon (after 10 a.m. to 12 noon), and afternoon (2 p.m. to 4 p.m.) when the female flowers are open and the stigma receptive. Data was collected on fruit set and percentage fruit was computed. There were varying percentages of fruit sets across the cross combinations and time of pollination. For the cross TDr110083 × TDr9501932, the highest fruit (55%) was recorded when pollinated at noon but when the female was pollinated with the second male (Fakesta), best fruit set (45%) was attained when pollination was done in the afternoon. However, when the two males were used to pollinate a second female (Akunchi), best fruit sets were observed in the afternoon. Variability of pollination success with timing of pollination was also noticed with *D. alata* crosses (data not shown). In general, this preliminary assessment confirmed better pollination success with noon and afternoon pollinations as has been reported with previous studies.

**Observation on parthenocarpic fruit development (Apomixis) in *D. rotundata*:** There are reports in the literature of parthenocarpic fruit development in yams in rare cases. This experiment was set up to assess the chance of apomixis (i.e., parthenocarpic fruit development) when polycross is used as an alternative crossing method to generate half-sibs. It consists of seven different treatments including spatial and physical isolation with net coverings. The experiment was established across screen houses and fields with profusely flowering male and female parents and observation on both flowering and fruit set were recorded. Preliminary observation showed flowering in five out of the seven treatments and only the treatments that were hand pollinated developed fruits (see in Appendix 1). This experiment will be repeated in 2019.

**Establishment and management of seedling progeny nursery:** At Ibadan, half-sib seedling progenies under direct field transplant was assessed. Fruits harvested from 2017 GWAS field were processed and sown in seed trays in families. A total of 3,198 seeds from 21 families were sown on trays in the screen house on 26 and 27 of April 2018. Days to germination and number of germinated seedlings per family were recorded. A total of 3,012 seeds germinated with 96% survival rate. The seedlings were later transplanted to the field on 6 July 2018. In all, 2,298 seedlings were transplanted. Appropriate management (weeding, fertilizer application, trailing, etc.) was done from time to time. The seedling nursery performance for the year 2018 was not too impressive. This was traced to the poor nature of the field. Of the 2,298 seedlings transplanted only 1292 survived. A total of 225 tubers were harvested from all the 21 families. The average tuber weight per family ranged from 4 to 53 g (Details showed in Appendix 1).

**Tuber family evaluation:** A number of tuber family evaluations and selections were made at Ibadan during the 2018 cropping season. The tuber family trials included full-sib and half-sib progenies from controlled hand pollination and open field natural pollination. The first set of tuber families comprised 10 *D. rotundata* and 11 *D. alata* families and these were sorted into two groups based on tuber weight ( $\geq 50$  g and  $< 50$  g). Tubers  $\geq 50$  g were bulked per family and established on the field in a single-rep family evaluation trial. Tubers with  $< 50$  g weight were bulked per family, further sorted into four distinct shapes (cylindrical, spherical, oval, and irregular) and established based on this on the field to evaluate the uniformity of shapes over two generations. Data was collected on family basis for YMV and YAD responses. At harvest, data was recorded on the tuber yield component traits: number and weight of the three tuber size categories. Traits on tuber characteristics such as

**Commented [JL35]:** Do these reports give some indication of the environmental conditions and circumstances of these rare cases? Locations?

**Response:** The reports did not give those details.

**Commented [JL36]:** what accounted for the loss of 700 germinated seedlings that weren't transplanted?

**Response:** Unknown cause but I suspect the deleterious mutation might be the cause for seedling death. Or might be some fungal disease. We will increase the engagement of pathologist in our seedling nursery evaluation and management to sort out the cause of the seedling death.

**Commented [JL37]:** This calls into question the earlier statement about appropriate management - surely with better management the survival rate would have been much higher

**Commented [JL38]:** Besides the selections made, what other learnings come from this study? Can it affect your choice of parents/crosses made in 2019? What about traits recorded? Which ones have high  $h^2$  and are most reliable for selection at this early stage? Can you achieve similar results with less work? What traits

**Response:** The tuber family evaluation trial for the full-sib was unreplicated family selection trial and not arranged in such way to assess the heritability of traits at this stage. The intention of sorting the seedling tubers based on tuber weight ( $\geq 50$  g and  $< 50$  g) was to determine if any plant with less than 50g tuber will produce high or comparable tubers with that of  $\geq 50$ g size. We noticed few progenies with  $< 50$  g produced comparable tuber yield but the frequency of selected plants in  $\geq 50$  g category is high. We will repeat this exercise to get more reliable information that help our selection decision at early stage. From the frequency of selection we learnt that those parents with good general combining ability contributed much to the individual advanced to next breeding stage. Yes we are strategically building parental breeding value estimation in our breeding program and apply this for parent selection for next season crossing block.



tuber appearance, cracks, shape, and thorniness were also collected. Visual selection was made at harvest on the field. The visual observation resulted in the selection of 125 progenies out of 703 total progenies harvested.

Among the 10 families of *D. rotundata* full-sib families, the highest proportion (20%) of progeny selection was from Family TDr1655. The least proportion (4%) of progenies selected was from Family TDr1632 with five progenies selected. A total of 136 progenies were harvested from the > 50 g groups while only 28 progenies were selected representing a selection intensity of 20%. Family TDa1608 with 11 selected progenies has the highest proportion (39.3%); Families TDa1614, TDa1622 and TDa162 had no selected progenies. The second set composed of 40 *D. rotundata* half-sib families generated through direct field transplant in 2017. A total of 3145 progenies from 40 families were established in a replicated family evaluation trial in three replications. Family based data collection have been done for NEP, YMV, and YAD up to three months after planting (MAP)

Harvest and postharvest data were recorded on number of tubers (big, medium, and small categories), weights, and number of the tuber categories. Further data on tuber shape, appearance, hairiness, and thorniness were collected from individual plants from the 40 families. Visual selection was done using a combination of tuber desirability, yield, and uniformity of multiple tuber tendencies. Mean squares of traits as shown in Appendix 1 revealed significant differences among the families with regards to YMV severity, number of big tubers, number of medium tubers, and number small tubers ( $p < 0.05$ ). The distribution of the shapes and appearance of tubers of the progenies from the 40 families are also presented. With respect to tuber shape, more of progenies have cylindrical shape (89%) while only two progenies were spherical (< 1%). Again, 56% of the progenies had hairy tubers while 11% had tubers with fully smooth skin. Only 1% of the total progenies had thorny tubers. Family TDr16187 had the highest number of progenies selected with 17 progenies selected from 105 harvested progenies, while the least selected progeny per family was one (TDr16183, TDr16200, and TDr1620). However, no progeny was selected from three families (TDr16173, TDr16174, and TDr16175)

**Preliminary performance evaluation (*D. alata*):** Tubers emanating from selections made from TDaTFE of year 2017 were established in a preliminary performance evaluation trial under Ibadan conditions in 2018. The trial comprised a two-rep RCBD trial of 87 clones with two plants per plot. Data at vegetative stage were recorded on NEP, YAD, YMV, and vigour at 3MAP. At harvest, tuber yield component traits including number and weights of tuber categories were recorded. Tuber yield was also computed for clones. The mean square analysis showed significant difference among the clones for expression of tuber yield, total tuber weight, total tuber number, number of big tubers, weight of big tubers, and weight of small tubers ( $P < 0.05$ ) as presented in Appendix 1.

**Development of genetic gain panel on *D. rotundata*:** Fifty-two *D. rotundata* clones were assembled from materials developed at different times during the course of yam breeding. They included trait progenitors and elite lines that were used to monitor the genetic gain achieved with breeding efforts by the program. The panel was established at Ago-Owu site in a single rep layout consisting of four plants per plot with each plot sequentially comprising three tuber portions of head, middle, and tail. Twenty-six traits were phenotyped while leaf samples were collected and genotyped. Days to flower initiation ranged from 51 to 131 days after planting for the established clones. About 14% of the clones had less than 100 days for flower initiation. Approximately 94% of the established clones had low to moderate vigor with just three clones having high vigor. Multiple tuber production potential was common among the clones considered as 46% had two to three stems per plant. Average disease rating (YMV severity score) ranged from two to four indicating absence of disease-free plants. YAD severity score showed no variation. Plant sex distribution showed 48% male, 50% female and 2% monoecious female clones. At seven months after planting, 75% of the clones had senesced completely.

Complete harvest data for seven clones and stands from plots of other clones were lost to theft incident prior to harvest. Hence, 45 clones were evaluated. From the available data, yield ranged from 0.18 to 24.31 t/ha. Tuber appearance ranged from fully smooth to rough with roughly 16% fully smooth, 33% thorny, 47% hairy, and 4% rough. Only 9% had heavy crack, which was not attributed to soil pathogens such as nematode as all clones were scored free of root knot, mealybug, scale insect, and *Scutellonema bradys*. We also assessed tuber quality traits

**Commented [JL39]:** So these were planted from field-maintained tuber seed? No clean seed available?

**Response:** Yes, we don't have clean seed available

**Commented [JL40]:** Were some of the stolen clones known to be more preferred? Is the choice of clones stolen also a useful data point?

**Response:** Stealing of yams occurred quickly and randomly especially at night or early hours of the morning. The thieves target materials that are easily accessible and have no time to choose which clones to harvest.

**Commented [JL41]:** i.e. scored as free from obvious symptoms, not free after microscopic examination or DNA testing.

**Response:** Yes. The score was as free from obvious symptoms, not free after microscopic examination or DNA testing

and found that above two-third (76.5%) of the clones had uniform tuber flesh color (head, middle, and tail regions). Nineteen clones constituting three landrace and 16 breeding clones showed moderate (score 2) to high (score 3) levels of oxidization after 60 min of observation. Dry matter content ranges from 21% in Akunchi to 37% in TDr9519177. Details of the results are presented in Appendix 1.

## 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.

### Primary Outcome 2:

Activity 2.1.2.1: IITA and JIRCAS signed a no-cost-extension agreement (Feb 2018–Jan 2019) to enable JIRCAS complete Year 4 harvesting, analyze their data, and submit a final comprehensive report.

## 3. Geographic Areas to Be Served

Provide the most updated list of countries and sub-regions/states that have benefitted or will benefit from this work and associated dollar amounts. If areas to be served include the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Areas to Be Served can be found [here](#).

Location	Foundation Funding (U.S.\$)
Nigeria (Abia, Benue, Ebonyi, Edo, FCT, Kogi, Kwara, Nasarawa, Niger, and Oyo )	\$6,210,000
Ghana (Ashanti, Brong-Ahafo, and Northern regions)	\$4,050,000
Benin (Borgou, Collines, Zou)	\$1,350,000
Côte d'Ivoire (Belier, Gbêkê, Hambol, N'Zi, Poro, and Tchologo)	\$1,890,000

## 4. Geographic Location of Work

Provide the most updated list of countries and sub-regions/states where this work has been or will be performed and associated dollar amounts. If location of work includes the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Location of Work can be found [here](#).

Location	Foundation Funding (U.S.\$)
Nigeria (Abia, Benue, Edo, Ebonyi, FCT, Kogi, Kwara, Nasarawa, Niger, and Oyo)	\$5,587,204
Ghana (Ashanti, Brong-Ahafo, and Northern regions)	\$3,261,568
Benin (Collines, Borgou, and Zou)	\$1,172,561
Côte d'Ivoire (Belier, Gbêkê, Hambol, N'Zi, Poro, and Tchologo)	\$1,465,798
Japan (Iwate Prefecture)	\$1,165,195
Guadeloupe/France	\$323,855

USA, Ithaca, New York	\$9358,050
Scotland, UK	\$165,769

5. 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.

Foundation support for the project

1. Regular monthly Skype meetings with the Program Officer had a very positive impact on project implementation.

Recommendation for improvement

6. Global Access and Intellectual Property

If your funding agreement is subject to Intellectual Property Reporting, please click the following link to complete an [Intellectual Property \(IP\) Report](#).

If not, please acknowledge by typing "N/A":       N/A      

To delegate permissions to another member of your project team or for any questions regarding the Intellectual Property Report, please contact [GlobalAccess@gatesfoundation.org](mailto:GlobalAccess@gatesfoundation.org).

7. Regulated Activities

Do you represent that all Regulated Activities<sup>1</sup> related to your project are in compliance with all applicable safety, regulatory, ethical and legal requirements? Please mark with an "X":

      N/A       N/A (no Regulated Activities in project)

       Yes

       No (if no, please explain below)

Are any new Regulated Activities<sup>1</sup> planned which were not described in any documents previously submitted to the foundation? Please mark with an "X":

      X       No

       Yes (if yes, please explain below)

<sup>1</sup> Regulated Activities include but are not limited to: clinical trials; research involving human subjects; provision of diagnostic, prophylactic, medical or health services; experimental medicine; the use of human tissue, animals, radioactive isotopes, pathogenic organisms, genetically modified organisms, recombinant nucleic acids, Select Agents or Toxins ([www.selectagents.gov](http://www.selectagents.gov)), Dual Use technology ([http://export.gov/regulation/eg\\_main\\_018229.asp](http://export.gov/regulation/eg_main_018229.asp)), or any substance, organism, or material that is toxic or hazardous; as well as the approvals, records, data, specimens, and materials related to any of the foregoing.

Financial Update

The purpose of the Financial Update section is to supplement the information provided in the "Financial Summary & Reporting" sheet in the foundation budget template, which reports actual expenditures and projections for the remaining periods of the grant. This section is a tool to help foundation staff fully understand the financial expenditures across the life of the project. Together, the Financial Update section and budget template ("Financial Summary & Reporting" sheet) should provide a complete quantitative and qualitative explanation of variances to approved budget.

Note: If you are using an older version of the budget template, this information could be in a different location in your template.

## 1. Summary

Briefly describe how total project spending to date compares against the budget and how your assumptions may have changed as the project progressed.

The training of project accountants of our NARS partners which was done in the previous year resulted in timely reporting by all partners. An overview of our budget implementation reflects that budget line items in the category of personnel and travel were slightly above the planned budget. However, subgrants, consultancy and other direct cost were within the planned limits in the year after the re-classification of expenditures to correct budget line items since the inception of the project. The RTS charges in 2017 and the subsequent budget reforecast for Year 4 resulted in a significant reduction in the budget for Other Direct Cost to US\$150,183.15 for Year 4 operations. All project activities planned for the reporting period were however executed.

The forecast for capital expenditure was US\$4908 as against the actual expenditure of US\$9299 resulting in an over-run of 89%. This was mainly due to the charge of US\$8,959.83 being the cost of purchase of a 15 KVA generating set for use by the Project Leader in Abuja. The equipment was purchased in 2017 and is now posted to the journal causing an over-run of the budget line item.

Overall, funds usage by partners is proportionate to project activities carried out and shows effectiveness in performance.

**Commented [JL42]:** We haven't received the details of what exactly is included in these RTS charges, including names of individuals paid, percentage of time, specifics. These need to be reported transparently and accurately.

**Response:** The RTS charges were mainly additional overhead charges incurred as a result of inflation and general high cost of water, electricity and other tariffs paid by IITA and not funds paid to individuals. The details of these charges is attached to this report.

## 2. Latest Period Variance

Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether or not approval for the variance has been obtained from your Program Officer.

Note: "Latest period variance" compares actuals to previous projections for the period. See "Financial Summary & Reporting" sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

### Personnel

Overall, the project personnel spending was 14% above the planned budget. Dr David De Koeper left IITA and the project at the end of his contract term in April 2018. Dr Asrat Amele was appointed in June 2018 to replace him as IITA yam breeder with 100% of his time in AfricaYam. Furthermore, Mr Olubunmi Olukotun was appointed as Project Administrator to replace Mr Olurotimi Famodile who resigned in December 2017. Dr Agre Partene was also appointed as an associate scientist in December 2018. The slight increase in personnel spending was due to the newly appointed personnel.

### Travel

The Year 4 travel report shows 114% of the budget was spent within the reporting period. This includes travel costs of participation in conferences, workshops, and support visits to partners. The over-expenditure is largely due to advance travel bookings (flight tickets and hotel) for IITA staff and TAC members participation in the Year 5 annual planning meeting scheduled to hold in March 2019 in Abuja, Nigeria.

### Sub-grants

Following the approval of an NCE for the project, deductions from national partners were implemented as planned to ensure availability of funds for the NCE period. NRCRI, EBSU, UAC, CSIR-SARI, CSIR-CRI, and CNRA deductions were retained at IITA. BTI in its efforts to support capacity building of project team conducted a YamBase training workshop for 27 technicians across partners in Benin Republic, Cote d'Ivoire, Ghana, and Nigeria, and also supported the training with US\$19,600.

### Capital Equipment

Most of the capital items were purchased during the first three years of the project. There was no major capital expenditure in the current year. The forecast for capital expenditure was only US\$4908 as against the actual

expenditure of US\$9299, resulting in an over-run of 89%. This is mainly due to the charge of US\$8959.83, cost of purchase of a 15 KVA generating set for use by the Project Leader in Abuja. The equipment was purchased in 2017 and now posted to the journal causing an over-run of the budget line item.

#### **Consulting**

The JHI funds were paid for Year 4 although their budget still reflected a significantly low spending rate due to little or no activities assigned to them. JHI now planned a three-day training on “R” statistical package at Abuja in April 2019 and all NARS representatives are expected to be in attendance. The JHI funds which were initially classified as sub-grant since inception were transferred to the correct budget line (consultant)

#### **Other Direct Costs**

Other direct-cost line items was within the planned budget for the current period after re-classification of expenditures to the correct budget lines. Due to the amount of overspending in the previous years (largely as a result of increased breeding activities in IITA and the RTS charges of 2017), the Other Direct Cost has been reforecast for both period 5 and 6. This is to ensure project activities are delivered effectively, data captured, and report finalized.

#### **Indirect Costs**

In all, 97% of the indirect-costs budget was charged within the reporting period. This is within the project provision for the period under reference.

### **3. Total Grant Variance**

**Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether or not approval for the variance has been obtained from your Program Officer.**

Note: “Total grant variance” compares actuals plus current projections to the budget. See “Financial Summary & Reporting” sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

There are slight changes in the Year 5 and NCE budget reforecast for each category as compared with the earlier forecast. Adjustments for Year 5 and NCE budget reforecast have been made with the figures from the final Year 4 Annual Report. Details of the updated reforecast budgets for Year 5 as well as provision for NCE savings follow.

## **PERIOD 5 BUDGET RE-FORECAST**

Budget Category	Original Project Budget	Prior Period Forecast	Current Forecast
Personnel	733,460	620,660	619,387
Travel	109,566	44,607	90,000
Sub-grants	796,361	796,361	626,939
Capital Equipment	-	-	-
Consulting	36,708	55,644	25,644
Other Direct Costs	141,991	77,384	153,568
<b>Direct Costs, Total</b>	<b>1,818,085</b>	<b>1,594,656</b>	<b>1,515,537</b>
Indirect Costs, Others	-	-	-
Indirect Costs, Gates Foundation	269,077	235,829	224,300
Indirect Costs, Total	269,077	235,829	224,300
<b>Total</b>	<b>2,087,162</b>	<b>1,830,486</b>	<b>1,739,837</b>



## PERIOD 6 NCE BUDGET RE-FORECAST

Budget Category	Original Project Budget	Prior Period Forecast	Current Forecast
Personnel	-	73,510	80,278
Travel	-	21,707	20,392
Sub-grants	-	-	133,686
Capital Equipment	-	-	-
Consulting	-	19,984	14,984
Other Direct Costs	-	16,218	21,804
<b>Direct Costs, Total</b>	-	<b>131,418</b>	<b>271,144</b>
Indirect Costs, Others	-	-	-
Indirect Costs, Gates Foundation	-	19,770	40,129
<b>Indirect Costs, Total</b>	-	<b>19,770</b>	<b>40,129</b>
<b>Total</b>	-	<b>151,188</b>	<b>311,273</b>

### 4. Sub-awards (if applicable)

Use the chart to provide the name(s) of the sub-grantee(s) or subcontractor(s), actual disbursement for this reporting period, total disbursement to date from the primary grantee to sub-awardee, total spend to date by the sub-awardee and total contracted amount.

Note: The total of actual disbursements for this reporting period should equal the actual Sub-awards expenses reported on the "Financial Summary & Reporting" sheet in the foundation template for this reporting period. If you are using an older version of the budget template, this information could be in a different location in your template.

Organization Name	Actual Disbursement for this Reporting Period (U.S.\$)	Total Disbursed from Primary Awardee to Sub to Date (U.S.\$)	Total Sub-Awardee Spent to Date (U.S.\$)	Total Contracted Amount (U.S.\$)
Boyce Thompson Institute (BTI)	135,743	549,560	512,797.64	612,460
Iwate Biotechnology Research Center (IBRC)	168,540	1,103,887	1,103,887	1,164,264
Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)	51,712	253,974	253,974	311,659
Ebonyi State University (EBSU)	29,717	154,105	153,505	187,559
Centre National de Recherche Agronomique (CNRA)	114,706	559,564	551,257	695,423
National Root Crops Research Institute (NRCRI)	117,757	732,231	730,310	856,354
CSIR-Crops Research Institute (CRI)	90,320	540,821	468,939	667,166

Universite d'Abomey-Calavi (UAC)	70,237	387,856	387,856	470,382
CSIR-Savanna Agricultural Research Institute (SARI)	52,789.91	264,435	264,435	327,374
Japan International Research Center for Agricultural Science (JIRCAS)	131,356	425,499	348,073	425,500

5. Other Sources of Support (if applicable)

List and describe any sources of *in-kind* project support or resources received in the reporting period.

Note: Names of the other sources of funding and their contributions (U.S.\$) should be included in the budget template on the "Financial Summary & Reporting" sheet in the foundation budget template in the Funding Plan table. If you are using an older version of the budget template, this information could be in a different location in your template.

Describe how interest earned and/or currency gains were used to support the project.

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For Foundation Staff to Complete

Analysis (required if contingent payment or PO assessment differs from grantee/vendor assessment)

Progress Analysis

Include analysis of significant project variances and key learnings that may inform portfolio discussions for progress against the strategic goals.

Budget and Financial Analysis

Include analysis of unexpended funds or over expenditures. Refer to the [Unexpended Grant Funds Policy](#) for options available when recommending how to handle unexpended grant funds, or reach out to your primary contact in GCM.

Scheduled Payment Amount	\$
Carryover Amount	\$
Recommended Payment Amount	\$

Approver Comments (if applicable)

Name	Title	Date Approved

Comments