BILL & MELINDA GATES foundation

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	Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa		
Grantee/Vendor	International Institute of Tropical Agriculture		
Primary Contact	Rony Swennen	**Investment Start Date**	October 1, 2014
Feedback Contact[1]	Rony Swennen	**Investment End Date**	August 31, 2019
Feedback Email[1]	rony.swennen@kuleuven.be	**Reporting Period Start Date**	October 1, 2016
Program Officer	Jim Lorenzen	**Reporting Period End Date**	September 30, 2017
Program Coordinator	Emily Zuberi	**Reporting Due Date**	October 31, 2017
Investment Total	$13,873,600.00	**Opportunity/Contract ID**	OPP1093845
Scheduled Payment Amount (If applicable)	$2,780,258.00		

[1] 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	[October 31 2017]	**Submitted by Contact Name**	Allan Liavoga
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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.

Background:

1. This project focuses on drastically improving the speed and efficiency of breeding bananas, in particular starchy staple food bananas in Eastern Africa: it aims to dramatically upscale existing breeding activities, build a breeding and selection pipeline, improve data management, and increase the pace and efficiency of breeding by conducting research for identifying methods for achieving higher rates of seed set and developing molecular markers for early selection of priority traits. We will combine genetic and genomic studies on segregating populations for mapping sources of resistance to the target pests and diseases. This will be complemented by improved characterization of the spread and virulence of the four target pests and diseases in five testing sites and the development and application of faster bio-assay screens. Also we develop a system for better tailoring breeding products and increasing adoption of new cultivars through end-user feedback systems and participatory evaluation of improved germplasm. All information obtained is deposited in an open-source database to improve breeding efficiency and intensify internal collaboration.
2. Structure of the project: structured around five strategic goals and one management goal:

1. **Banana Breeding Pipeline:** Enhanced performance of breeding systems to deliver improved East African highland bananas with increased levels of pest and disease resistance, higher yields, and better consumer acceptability.
2. **Pest and Disease Control:** Enhanced host plant resistance to major pest and disease constraints through improved pathogen identification and accelerated early stage screening of resistance.
3. **Leveraging Genetics:** Improved breeding efficiency through molecular-based genetic studies for increased understanding of underlying genetics and development of DNA marker-based early selection.
4. **Empowering End-user Evaluation:** System for better tailoring breeding products and increasing adoption of new cultivars through end-user feedback systems and participatory evaluation of improved banana germplasm.
5. **Harnessing Data:** Driving improved efficiency of breeding systems and enhanced synergy in national, regional, and global partnership through an open-source database and tool box for banana breeders and researchers.
6. **Governance, Research Oversight, and Management**: Coordinating breeding efforts integration, capacity building, communication, and dissemination undertakings and to ensure long-term project impacts, through embedding breeding and research planning, and review in a users' perspective forcing all in the research-for-development process into an adoption-orientated focus.

Executive Summary

During Year 3 we are very pleased to report that project implementation has effectively progressed on track, if not ahead of our ambitious targets in some areas. Numerous hybrids have been identified with various resistance to targeted pests and diseases, with good survival and bunch weights. From EETs 190 Matooke hybrids have been selected for multiplication for PYT and 74 Matooke well phenotyped hybrids from the training population. Phenotyping of the mapping populations has also made good progress, including the clarifications of some errors from previous work. In combination with the use of SSR markers 526 accessions, representing several mapping populations, were genotyped during this period and cross checked for their true-to-typeness. Noteworthy is that Mchare/ Muraru/ Mlali accessions from Tanzania and Kenya all group together. To date >11,000 hybrids of various crosses have been developed, many already in EET, including nearly 500 Mchare-2x crosses and nearly 200 hybrids from 3x-2x crosses. The cumulative number of embryos cultured amounts to 1581 from 3x - 2x crosses and 122,784 from 4x - 2x crosses and in Mchare breeding, 1,650 seeds, over half the number promised for the project, have been obtained. Studies to assess breeding success, pollination and fertilization is providing much needed insight towards increasing efficiency as well as end-user traits even. For example, together with in depth phenotyping in the field, results show high grandparent heterobeltiosis for almost all NARITA hybrids for bunch weight. Female fertile cultivars and hybrids have been identified, for high priority/preferred use in crossing. It is now also clear that seed set capability depends on pollen viability and quantity, but importantly that it is a combination of the two, as well as time of day. Pollen viability varies during the day and also within the month, unlike pollen quantity. The application of sucrose on stigmas to increase seed set is also better understood and its success now known to be dependent on certain times of the year. Numerous pest and disease populations have been collected during the year in order to map distributions and characterize species and strains. The presence of pests and diseases depends on the location, altitude and variety. More data is available for Foc and Sigatoka than nematodes and weevils, although links with N. Luambano (PEARL Project) contributes significantly for information on nematode data. Assays for optimized screening have been developed, including an improved Foc infection/inoculation method, and with good progress made with field and screenhouse trials established in Tanzania and Uganda. Differences in Mchare nomenclature between Tanzania and Uganda creates difficulties for comparing results at the moment. Progress continues in genotyping mapping populations to study segregation for Fusarium, nematodes, weevils and Sigatoka plus other traits. Segregation for Foc R1 was confirmed in Monyet x Kokopo, Calcutta 4 x Calcutta 4 and Paliama x Borneo, while phenotyping for Foc SR4 and TR4 continues in Australia. Following inoculation with a subtropical Foc Race 4 strain (VCG0120) gene expression levels indicated 2 candidate genes that were significantly up-regulated in resistant Malaccensis. Furthermore, a QTL for Foc SR4 has been identified on chromosome 3. The QTL has been converted into a PCR-based marker and confirmed in known Malaccensis resistant genotypes. DNA for 18 hybrids was sent to UQ for genotyping for the Foc SR4 QTL and resistance against Foc TR4 should be expected from some of these. Of six genomic selection models being assessed, using training populations under contrasting management, BayesB model appears superior, which will be further fine-tuned using the phenotypic data from Mbarara, a third location, and using data of the third cycle in all 3 sites. IITA and NARO have begun the leaf archiving of all genotypes being phenotyped, with 2688 genotypes so far archived. From EMBRAPA, 20 parthenocarpic diploids have been received as well as 62 diploids with *banksii* background from ITC, for cataloguing and potential use in breeding.

A wealth of information has been compiled for products linked to 4 banana types from 5 testing sites, which has identified the varietal performance to deliver either a good or bad product. It also became clear that a certain postharvest characteristic (trait) can also be linked to the status both before or after processing. A systematic review on gender-differentiated banana trait preferences across the value chain shows a large range of preferred traits by households, which can be grouped into five main categories for each of the banana types (Physical descriptors; Sensory/organoleptic descriptors; Processing and product related traits; Socio-economic descriptors and Others). Cooking bananas appear to fulfill the highest number of these characteristics, followed by plantain. These studies will be helpful in preference ranking of NARITAs. The banana trait ontology tool has been updated, and an additional tool developed to allow the building of own traits, has now been uploaded to the Crop Ontology website, while field trial information was uploaded into MusaBase and mobile data from the field sites hosted on a newly developed server. NARITAs are being multiplied for on-farm trials in 2018. A workplan has been developed for a gendered analysis of the seed systems in target regions for adoption of the NARITAs, which is linked to RTB cluster CC2.1 and a new project proposal RTB FOODS. To help track seed, monitor progress and report results in banana breeding a banana cross tracking tool has been developed using a standalone application on a handheld device that will become interconnected to MusaBase.

During Year 3 four new partners (IEB- Institute of Experimental Botany, Czech Republic**;** UNCC- University of North Carolina at Charlotte, USA; DHMRI- David H. Murdoch Research Institute, USA; and WCMC- Weill Cornell Medical College, USA) were welcomed on board to support genotyping and SNPChip activities, and relations with existing partners strengthened and improved through interactions, germplasm exchange and breeding team exchange visits. The physical infrastructure in Arusha and Sendusu have been significantly upgraded to support project research. The annual meeting was successfully undertaken in Kampala and the website formally launched, creating greater visibility of the project, while a series of publications, presentations and media outputs provide additional visibility and demonstrate project progress. An IITA internal audit and BPAT review were conducted, both of which will help to identify strengths and

weaknesses within the project, to build upon or address accordingly, while FAO and the World Banana Forum have invited IITA to lead Musa breeding in the Global Program on Fusarium Wilt Disease.

3. Organization of the strategic research goals:
   Part A. The first 5 strategic goals are organized around 5 work packages (WP), while strategic goal 6 is implemented via a project leader, a steering committee, and a science advisory group.

   1. Banana Breeding Pipeline: led by NARO, in collaboration with IITA, KULeuven, EMBRAPA, and NRCB.
   2. Pest and Disease Control: led by Univ. of Stellenbosch (South Africa), in collaboration with Univ. of Queensland (Australia), NARO, IITA, ARI (Tanzania), and Bioversity International.
   3. Leveraging Genetics: led by IITA, NARO, SLU-Sweden, in collaboration with Univ. of Stellenbosch, Univ. of Malaya, Univ. of Queensland, EMBRAPA, IEB, UNCC, DHMRI, and WCMC
   4. Empowering End-user Evaluation: led by Bioversity International, in collaboration with ARI-Tengeru, NARO, and IITA.
   5. Harnessing Data: led by BTI, Cornell Univ., in collaboration with IITA, NARO, NRCB, and EMBRAPA.

   Part B. Status of Contracts and consequences:
   1. We are pleased to report that interactions with the UM have intensified and that a joint PhD has started.
   2. NRCB, India. It was mutually agreed to conduct transactions through a contract between IITA and Bioversity Office in New Delhi signed by contract amendment on 12 October 2016. One NRCB staff participated at the annual meeting in April 2017 in Uganda and NARO and IITA banana breeders visited NRCB in August 2017 to facilitate progress and prepare the work plan for the next 2 years.
   3. A contract with IEB was signed to provide continuous support in genotyping all material throughout the work as to assure trueness to type.
   4. UNCC, DHMRI and WCMC were contracted in support of the production of a banana SNPChip.
   5. In the Results Framework and Results Tracker we provide information on the progress of each contract and variation of each output, and presented based on primary outcomes 1–9.
      a. primary outcomes 1: WP on Banana Breeding Pipeline
      b. primary outcomes 2: WP on Pest and Disease Control
      c. primary outcomes 3: WP on Leveraging Genetics
      d. primary outcomes 4–7: WP on Empowering End-user Evaluation
      e. primary outcomes 8: WP on Harnessing Data
      f. primary outcomes 9: WP on Governance and Management

      This will be further discussed in detail, with additional details provided in Annexes 1-5.

4. Organization of the Governance, Research Oversight, and Management:
   1. Management: there has been no change in the Project Management team, consisting of Project Coordinator and technical lead: Rony Swennen; Project Manager: Danny Coyne; Project Administrator, Scola Ponera; who work closely as a team. Significant activities during Year 3 have included organizing the Annual Project Workshop, establishing the project website and coordinating SC/Management meetings and SAG interaction with Project members.
   2. Steering Committee (SC): With the departure of Ylva Hillbur from IITA, the IITA Director for Eastern Africa, Dr Victor Manyong, was nominated to act as Chair of the SC, which he accepted. The SC: consisting of nine members including the secretary and a BMGF non-voting member met at the Annual Project Workshop in a separate and then a combined meeting with the Science Advisory Group (SAG). There have been no changes to the SAG since the annual report for Year 2 (see Annex 1.1.1).
   3. SAG: All members, except 1, attended the Project Annual Meeting in person. The SAG has been in contact with WP leaders and staff on an *ad hoc* basis and reviews project outputs against the existing Results Framework and Results Tracker. At the annual meeting the SAG recommended the inclusion of a breeder, preferably from the private sector, as an additional member and an updated vision and more strategic discussion towards impact on farmers in the long term. They appreciate the team spirit and interaction but emphasize for communication within the team and outside of (Annex 1.1.2-3).
   4. Annual Project Workshop was held successfully in Kampala 24-27th April 2017, which included visits to NARO Namulonge and Sendusu (Annex 1.1.2).
   5. The anticipated African Development Bank (AfDB) initiative with IITA: Technologies for African Agricultural Transformation (TAAT) has been initiated but has delayed the involvement of banana and plantain value chain until 2019.
   6. FAO and the World Banana Forum invited IITA to play the lead role in Musa breeding in the Global Program on Fusarium Wilt Disease, made public on 13 October 2017 (Annex 1.2.13-15).
   7. IITA undertook an Internal Audit of the project in August-September, covering the period from the 1st of January 2016 to the 30th of July 2017. The overall objective of the audit was to evaluate the adequacy, effectiveness and efficiency of the governance, risk management and internal control systems over the project. The report has yet to be provided, but preliminary highlights include: project implementation level above the mid-term target; good interactions and common understanding of the mission of the project among all stakeholders; good monitoring of financial transactions within IITA through periodic sharing of postings by the Project Account Officer; investment by the project in the refurbishment of the lab facilities at Sendusu; great synergy, team work and coordination among the project staff and the implementing partners; development of the project website for use as a marketing tool and linking with the global banana database, MusaBase. A number of risk issues were also identified, the key ones of which revolved around delayed reports to IITA, their approval and consequent delay of funds to partners, in particular NARO and ARI, as well as the lack of risk management processes. The report will be used to build on the highlights and improve shortcomings.

5. Capacity building and communications:
   1. Website: The project website framework was finalised and the site launched, which provides a resource platform both for the general public as well for project members through password protected entry to restricted areas. The website links directly with MusaBase as well as other relevant domains, such as RTB, IITA, in addition to all partner websites, while social networks (i.e. Facebook, Twitter, LinkedIn, Pinterest) are also linked in. The site is being continuously updated, information added, and data and documents being made available. Early statistics show that most of the access is from new users (Annex 1.1.4).

2. A total of 16 postgraduate students are currently associated with the project as shown in Table 1, 2 and Annex 1.7, similar to Year 2. Capacity building is a key objective of the project and it aims to increase the number of associated students, using the project as a platform to support those with alternative funding for training and thesis work, especially female students.

Table 1.

	Female	Male	Total	Supported by the project	Support from outside the project
PhD	1	8	9	7	2
MSc	1	6	7	2	5

Table 2.

Work package					
	WP 1	**WP2**	**WP 3**	**WP 4**	**WP 5**
PhD	5	2	2	0	0
MSc	3	0	2	1	1

3. During the year a range of outputs have been produced and communicated through various channels (Annex 1.1.5). The Annual Workshop in April and the official launch of the refurbished Laboratory at Sendusu IITA in Uganda at the 25 year celebration of IITA in Uganda achieved major exposure through television, radio, and newspaper, as well as through the many attendees, including numerous prominent Ugandan officials. A total of 9 book or journal articles were produced during this period by project staff/partners and at least 7 are in process of review, drafting or being planned. Twelve oral conference presentations were made and 13 poster presentations made at international meetings, including at the FAO convened Global Banana Forum, plus 15 at the annual project meeting. Some of this was funded by other projects but results are relevant for this project. In addition, a number of training workshops have been conducted, especially in relation to MusaBase and data entry.
4. Further to the SC and SAG meetings at the Annual Meeting in Kampala a schedule for management meetings on a two-monthly basis via skype has been established and maintained for regular contact between WP leaders and SC, an attribute highlighted by the internal audit towards positive interaction and team spirit.

6. The investment outputs and outcomes: the major achievements are:

Primary outcomes 1: WP 1 on Banana Breeding Pipeline

Scope and Approach: Enhanced performance of breeding systems to deliver improved East African highland bananas with increased levels of pest and disease resistance, higher yields and better consumer acceptability.

The goal of this work package is to increase breeding efficiency.

Therefore the objectives are (now reworded from the submitted project document):

1. Upscale the breeding activity;
2. Increase efficiency by obtaining higher seed set and higher numbers of hybrids;
3. Development of an international breeding platform.

Objective 1: upscaling breeding

We are reaching our ambitious targets in delivering the expected quantity of material (95 promising Matooke hybrids by year 5). Most importantly we have selected 190 Matooke hybrids so far from EET and these are being multiplied for PYT (Annex 1.2.1). A bonus came from the research on developing genomic prediction models (see WP 3) as 74 Matooke hybrids were additionally selected and phenotyped as part of the training population (Annex 1.2.2); below information coming from field observations over 3 uncompleted cycles:

- 18 hybrids, which combine good bunch weight (better than the local check), tolerance to black Sigatoka (INSLF between 67-86%), survival rate of 83-100%, and yields ranging from 16-26 t/ha/year;
- 21 hybrids whose bunch weight is comparable to that of the local check but having a higher survival rate of 83-100% and tolerant to black Sigatoka (INSLF ranging from 68-72%), resulting in yields of 10-17 t/ha/year;
- 3 hybrids with bunch weights better than the local check and survival rate of 83-100% but as susceptible to black Sigatoka as Enzirabahima (INSLF of 65-68%), yields from 15-26 t/ha/year;
- 16 hybrids with a bunch weight better than the local check but with a survival rate ranging from 33-67%, and more tolerant to black Sigatoka than the local check, thus promising a good yield in the first crop years;
- 1 hybrid with a bunch weight and tolerance to black sigatoka comparable to the local check but with 100% survival rate, resulting in a yield of 14 t/ha/year.
- 5 hybrids with bunch weights comparable to the local check, with a moderate survival rate (67%) but more tolerant to black Sigatoka than the local check, giving a yield of 9-10 t/ha/year. These hybrids are only of use to continue to develop the genomic prediction models not for further testing.

In Mchare breeding, major progress has been made, obtaining 1,650 seeds out of the 2400 targeted seeds promised (table below).

Other key achievements (table below) with cumulative data over the past 3 years are:

6343 hybrids from 4x-2x crosses, already planted in EET
4687 hybrids from 2x-2x crosses,
193 hybrids from 3x-2x crosses and already planted in EET
474 hybrids from Mchare-2x crosses in EET.

There are currently at least 1,500 seedlings in screen houses at IITA and NARO generated from across all ploidy levels that are yet to be planted into early evaluation trials.

The cumulative number of embryos cultured during the first 3 years of project = 1581 from 3x - 2x crosses and 122,784 from 4x - 2x crosses.

Objective 2: Increase efficiency by obtaining higher seed set and higher numbers of hybrids

A study was undertaken to analyze breeding success of Matooke varieties over the past 20 years (Annex Annex 1.7.1 [Batte]) . Some preliminary results show that cv Nakabululu had the highest pollination success (34.3%) and an average of 1.5 seeds per pollinated bunch, followed by Nakawere with pollination success of 31.6% and average of 1.4 seeds per pollinated bunch, Nakasabira with pollination success of 20.6% and average of 1.2 seeds per pollinated bunch and Nakyetengu with pollination success of 15.8% and average of 1.2 seeds per pollinated bunch. These were the highly female fertile cultivars and therefore highly recommended (high priority) for use in crossing. We currently have 15 Matooke varieties (Bitambi, Entukura, Enzirabahima, Nakyetengu, Kabucuragye, Kazirakwe, Nakabururu, Nakasabira, Nakawere, Nakayonga, Namande, Namwezi, Nfuuka, Rwambarara and Tereza) growing in 6.0 ha pollination blocks at IITA-Sendusu and NARO-Kawanda. Based on seed set, it is tempting to select just a few varieties for future breeding with the highest seed set but given our limited knowledge of end-user acceptability of hybrids (and limited knowledge of inheritance of key agronomic/fruit traits (especially plant height, suckering, bunch size and shapes, and fruit DM content, texture, and color)) and awaiting the outcome of such work, which is part of WP 4, we maintain those fields. Besides, current end-user acceptability understanding of NARITA hybrids remains tentative (Annex 1.2.3) as it was performed on a limited scale, and conflicts partly with an earlier report of NARITA response. Consequently as part of WP 4 and the upcoming project RTB Food, the NARITA hybrids will be properly documented for end-user value and postharvest behavior. Consequently, NARITA hybrids in the multilocational program (see WP 4) were phenotyped in-depth (Annex 3). In addition NARITA hybrids are being analyzed for heterobeltiosis (Annex 1.7.1 [Batte[) with all plants coming from the same field. About 90% of data for cycle 1, 75% for cycle 2 and 45% for cycle 3 have been collected. Preliminary results show high grandparent heterobeltiosis for almost all NARITA hybrids for bunch weight, with NARITA 9 having the highest value (201.6%) and NARITA 19 having the lowest value (5.3%). This implies that the parental combinations that produced the NARITA hybrids are good for producing hybrids with better bunch weights than their grandparents.
Similarly, hybrid performance was studied across all years available. Genotype 1201K-1 exhibited the highest pollination success (48.4%) with an average of 29 seeds per pollinated bunch, followed by 917K-2 with pollination success of 48.2% and average of 39.2 seeds per pollinated bunch, 660K-1 having a pollination success of 43.3% and average of 14.8 seeds per pollinated bunch and 222K-1 having a pollination success of 40.9% and average of 16.6 seeds per pollinated bunch. These tetraploid parents were the most female fertile and therefore recommended for preferential crossing. As we await postharvest information, we will not change the fields of the 10 Matooke tetraploids in the large pollination blocks (1.0 ha) at NARO-Kawanda consisting of 917K-2, 660K-1, 222K-1, 401K-1, 376K-1, 1201K-1, 1411K-1, 199K-3, 1438K-1 & 365K-1. We investigate the combination of high seed with high progeny value, but if not feasible give preference to high progeny value and rather work with a low fertile female. Quality prevails over quantity.

Breeding relies on the use of improved diploid hybrids. Therefore the catalogue of improved diploids was further completed (Annex 4) and 20 parthenocarpic diploids received from EMBRAPA multiplied *in vitro* and will be planted at IITA-Sendusu in October 2017. Also, 62 diploids with *banksii* background were obtained from ITC.

Pollen quantification and pollen viability studies have now been initiated both at IITA Arusha and at NARO-Kawanda (Annex 1.2.4-5-6). It is clear that seed set capability depends on pollen viability and quantity, but ranking of most fertile males depend on the combined effect of both pollen viability and quantity Annex 1.2.6). In addition, pollen viability is maximal at 8 am, declining thereafter during the day, while pollen viability varies also within the month, unlike pollen quantity. This study is in support of increasing seed set.

Female fertility in Mchare indicated that the most popular varieties of Mchare Laini and Huti white are also the most male fertile varieties (Annex 1.2.6), while only Huti white is seed fertile with 1.8 seeds per pollinated bunch (Annex 1.2.7).

As breeding of banana is complicated by different genotypes flowering at different moments, crosses change over time. In addition the follow up of hybrids through the ripening chamber, embryo rescue, *in vitro* culture, nursery and field planting is extremely complicated. Therefore the Banana Tracking Tool was developed, a data management system that is complementary and will be fully integrated with Musabase (Annex 1.2.8). It is now tested at IITA Arusha and will, in 2018, be implemented at IITA Uganda and NARO-Kawanda.

Pictures of floral development in Matooke (Enzirabahima and Nakitembe) and Mchare (Mulelembo and Mshale) were compiled. Results indicate that bracts of seed fertile Matooke and Mchare varieties open earlier than those of seed sterile varieties; flowers of seed sterile varieties usually open in the evenings. Field studies on Matooke (Enzirabahima and Nakitembe) and Mchare (Mulelembo and Mshale) flowers, especially on the patterns of seed set have shown that the stigma can be more receptive before flower opening, while seed set occurs mostly in distal hands. Collection of pictorial data of floral development characteristics in Mulelembo and Mshale is ongoing.

In Year 2 we reported a seed set increase of 108% when stigmas were treated with sucrose. This statement needs to be mediated however, further to recent results, which shows that the positive effect of sucrose is only valid for those months when seed production through routine application is already above average. Hence the effect of sucrose appears to augment seed set only during a few months of the year (Annex 1.7.1 [Waniale]) but conclusions are preliminary due to low number of observations.

A leaf archive of hybrids has been developed and is reported in WP 3 as the leaf archive also contains mapping populations.

Objective 3: Build an international breeding platform

Progress in banana breeding requires collective and collegiate involvement with the best partners for strong, healthy and reliable collaborations across partners. Current progress has been further strengthened based on new results and on activities not previously planned. For example, UQ is now testing germplasm and hybrids from IITA, NARO and EMBRAPA for potential sources of Foc TR4 resistance (see WP 3) and EMBRAPA is now screening 24 new improved diploids, which have remained Foc R1 resistant after 15 months (Annex 1.2.9). These hybrids are being additionally screened against black Sigatoka. EMBRAPA has also adopted the use of the same SSR markers for fingerprinting as IEB.

IEB is fingerprinting germplasm and hybrids for true-to-typeness (WP 3) but also to identify relationships between genotypes to support decision making for crosses. In 2017 NRCB joined us, and a 2 year work program discussed. NRCB has received germplasm from IITA and will be a strong partner in breeding and screening for *Fusarium* resistance. NRCB is chromosome doubling their diploids but will now have access to an improved method that is being used in our project.

In 2017 we underwent an external review by the BPAT breeding team. Their report is still outstanding but remarks provided during several concluding meetings, allowed us to reconsider some operations (Annex 1.2.10). The most important point is the need for specialization between the 3 breeding sites to reduce redundancies in the production and evaluation systems. This will increase the overall efficiency of the program while allowing each team to specialize in one or more aspects of hybrid production. Arusha is the natural candidate for diploid improvement. Sendusu is to focus on tetraploid (3x X 2x crosses) production, Kawanda on secondary triploids (4x X 2x crosses). Yet we will keep some redundancy to deal with potential risks like wind damage, Fusarium...The BPAT team will meet with the banana and plantain breeding team on 27 November at IITA Ibadan.

We also designed a strategy to develop a SNPChip (Annex 1.2.11) involving KULeuven to produce the DNA of 20 genotypes, sequenced by Genomics Core Research Facility, Cornell Medical School, NYC, USA. Sequence data have been downloaded from vendor website and is currently housed at University of North Carolina at Charlotte's (UNCC) Biocomputing facility at Kannapolis, North Carolina, USA.

Our international breeding platform has focused on breeding East African cooking bananas, but is also linked to the IITA plantain breeding platform. Plantain breeding, which is a relatively small operation at IITA in comparison with the East African cooking banana breeding program, stands to gain significantly, while in return can offer excellent skills with *in vitro* culture, chromosome doubling, provitamin A characterization of germplasm and hybrids and phenotyping. Both teams meet every 6 months.

This breeding platform has also attracted the attention of FAO and the World Banana Forum, with IITA invited to play the lead role in Musa breeding in the Global Program on Fusarium Wilt Disease (Annex 1.2.12-13). This Program on Fusarium Wilt Disease was presented on 13 October 2017 (Annex 1.2.14-15).

Parameter	**Target**	**Actual overall numbers (after 2 years)**	**Actual overall numbers (after 3 years)**	**Expected results for the next 2 Years/ General remarks**
Seed increase in Matooke and Mchare	15-20%	3x-2x = 1,650 4x-2x = 113,153 Mchare-2x =1508	3x-2x = 2680 4x-2x = 170,236 Mchare-2x =1673	Seed increase is the result of more pollination blocks in use
Improved diploids integrated into the Matooke and Mchare breeding pipeline	70	30 improved diploids are being characterized and already integrated in the NARO-IITA breeding pipeline. 20 parthenocarpic diploids received from EMBRAPA are under multiplication.	32 improved diploids are being characterized and already integrated in the NARO-IITA breeding pipeline. 28 improved diploids have been identified as potential selections from EETs 20 parthenocarpic diploids received from EMBRAPA were multiplied in vitro and are to be planted at IITA-Sendusu in October 2017. 62 *banksii* diploids received from ITC and multiplied *in vitro* at NARL. They are weaned for planting at IITA-Sendusu.	Considering the available number of improved diploids being characterized (32) and yet to be characterized (28+20=48), it most likely that the total number of diploids to be integrated in the NARO-IITA breeding pipeline will exceed 70.
Matooke hybrid under evaluation in the EET (early evaluation trial)	12000	3008 hybrids from 4x-2x and 127 hybrids from 3x-2x crosses are in EETs.	6343 hybrids from 4x-2x crosses and 193 hybrids from 3x-2x are under evaluation in the EETs. Over 1000 seedlings generated from 4x-2x crosses from May-October 2017 are in the screen houses at IITA and NARO. Over 10,000 hybrid seeds generated during July to September 2017 are undergoing embryo rescue and more seeds are being generated.	At this rate we expect to reach the project target.
Matooke hybrids (beyond NARITA 1-26) tested in PYT (preliminary yield trial)	95	25 potential hybrid selections for advancement to PYTs are already identified from an EET of 930 genotypes. This is an estimated rate of success of selection of 2.7%.	Of the 190 hybrids selected from EETs, 105 are under *in vitro* multiplication for PYT to be planted by April 2018.	The target of 95 hybrids to be tested in PYTs is surpassed.
Development of Mchare hybrids	2400 seeds for embryo rescue		1,650 Mchare seeds are so far generated.	More Mchare seeds are continuously generated.

Primary outcomes 2: WP 2 on Pest and Disease Control

Scope and Approach: Enhanced host plant resistance to major pest and disease constraints through improved pathogen identification and accelerated early stage screening of resistance.

The goal of this work package is to provide the tools to faster screen parents and hybrids towards improved efficiency in banana breeding. Pests and diseases in order of importance are: Fusarium, nematodes and weevils, and Sigatoka.

Therefore the objectives are (now reworded from the submitted project document):

1. Establish collections of the 4 pests and diseases: in support of future screening of germplasm and development of molecular tools for diagnostics;
2. Determine the presence of the 4 pests and diseases in the testing and breeding sites: in support of field screening and genotype by environment effects and regional testing;
3. Develop diagnostic techniques for pests and diseases: for precise identification of the pest and pathogen;
4. Bioassay: for higher screening, more rapid throughput using younger plants;
5. Screen Matooke and Mchare (plus hybrids): to identify sources of resistance.

The stage of progress depends on the pests and diseases.

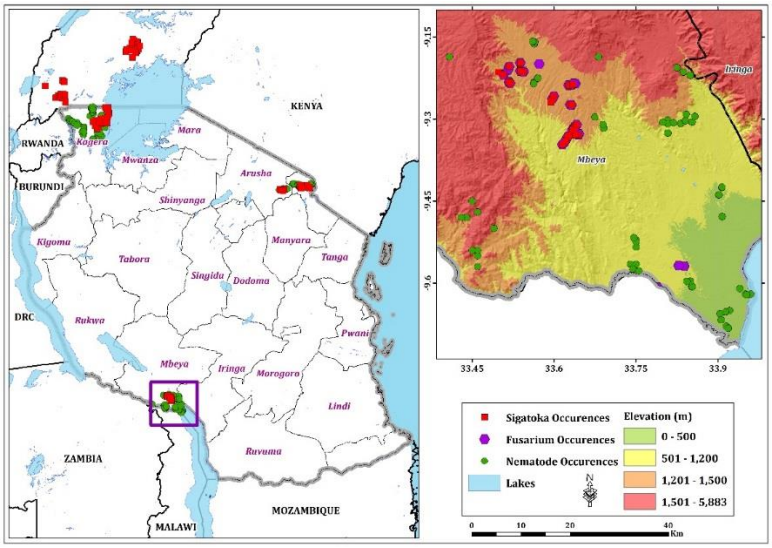
Objective 1. Collections of the 4 pests and diseases established.

Pest/disease	Location of storage	Number of samples
Fusarium	Stellenbosch University Department of Plant Pathology (USPP)	258 fungal isolates stored at -80°C. These isolates include Foc isolates, other Fusarium species and non-Fusarium species
Sigatoka	The isolates from Uganda are curated in IITA Pathology laboratory at Kawanda while those from Tanzania are stored at IITA laboratory at Nelson Mandela labs and Dar es Salaam.	200 pure isolates of *P. fijiensis* on glycerol stock at -80°C
Nematodes	Horti Tengeru	20 samples
Weevil	NARO	123 400 weevils from Uganda representing 8 populations (named by collection site)

All 3 breeding sites (IITA Arusha, IITA Sendusu, and NARO Kawanda) and 5 NARITA field testing sites, in addition to some other sites, were visited and Fusarium and Sigatoka samples collected. In the case of weevils, a survey was made in Uganda while no progress was made on nematodes. This brings the number of samples in the collection as shown in the table above.

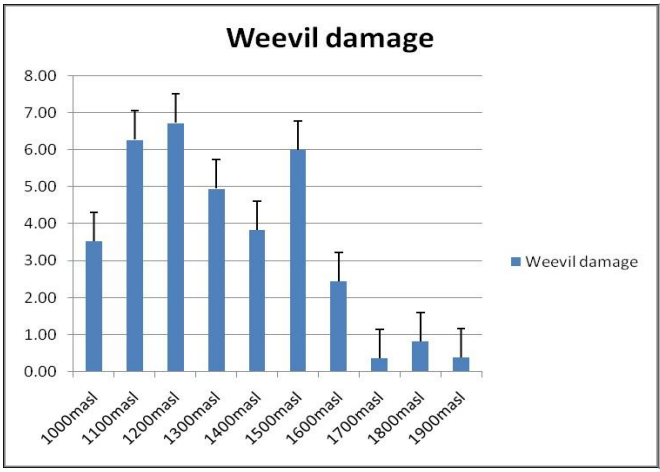
Objective 2. Testing and breeding sites screened for the presence of the 4 pests and diseases.

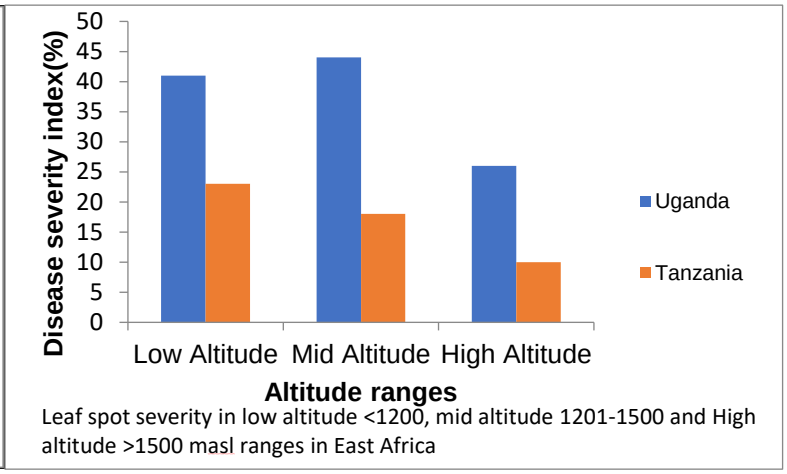
The distribution of pests and diseases of banana in East Africa has been completed. GPS coordinates of all samples at the five screening sites in Tanzania and Uganda were recorded. Maps were developed showing the presence of the 4 pests and diseases and presented in relation to location and altitude. For more maps we refer to Annex 1.3.1.



The presence of pests and diseases depends on the location, altitude and variety, as shown by the distribution of the VCG in Tanzania and Uganda (figure below and Annex 1.3.1, and 1.3.9). The banana weevil damage on East African Highland bananas decreases with increasing altitudes as of 1600 masl (Annex 1.3.2-3-4-5).

Sigatoka was more severe in Uganda than Tanzania. Disease severity and Sigatoka leaf spots were more severe in the lower and mid altitude ranges than higher altitudes, with no significant difference in disease severity observed between low and mid altitudes (Figure and Table below and Annex 1.7.1[Kimunye]). So far no Sigatoka has been recorded in Arusha.





Leaf spot severity in low altitude <1200, mid altitude 1201-1500 and High altitude >1500 masl ranges in East Africa

Country	District	Altitude (masl)	No farms surveyed	No. of samples collected	*P. fijiensis* positive samples
Uganda	Mbarara	1411-1877	18	152	97 (63.8%)
	Luweero	1077-1243	24	140	84 (60%)
	Kawanda	1182		30	30 (100)%
Tanzania	Bukoba	1148-1394	24	140	119 (85%)
	Mbeya	1064-1455	27	299	98 (33%)
	Arusha	1210-1530	17	159	0

Number and percentage of samples testing positive for *Pseudocercospora fijiensis* from six regions in Uganda and Tanzania

Objective 3. Diagnostic methodologies of pests and diseases

Of 258 isolates collected, 215 Foc isolates were identified to VCG level and 5 heterokaryon self-incompatible (HSI) isolates of Foc; 38 other *Fusarium* or non-*Fusarium* species were determined (Annex 1.3.6-7). All the newly identified isolates belong to VCGs and VCG complexes associated with Foc Lineage VI, such as VCGs 0124, 0125, 0128, 01212, 01220 and 01222. The Foc profiles therefore remain similar to that previously reported.

VCG 0124, 01222 as well as complex 0124/22 were recovered from five sites in Uganda and Tanzania, and represented 47.3% of all the Foc isolates collected. The VCG complex 0124/22 was dominant in Mbarara, Kawanda, Kagera and Arusha, and VCG 01212 in Mbeya. VCG 01212 and 01220 were not identified in the screening sites in Uganda. VCG 01220 was the least represented, with five isolates only collected at the Kagera site. Other Fusarium species, such as non-pathogenic *F. oxysporum* and *F. sacchari*, were isolated from banana at the five sites (Annex 1.3.7).

No diagnostic work was conducted for weevils and nematodes.

All Sigatoka isolates were confirmed as *P. fijiensis* using the species-specific primer pair MF137/R635 (Annex 1.7.1[Kimunye]). Mating type analysis using MAT1/MAT 2 primers revealed that 54% of the isolates belonged to MAT 1 while 46% were MAT 2. Further characterization using SSR markers and virulence assays is in progress.

Detection of the other *Pseudocercospora* spp. associated with Sigatoka is pending optimization of a molecular detection system. Reported primers failed to amplify and because of the lack of a positive control, it was difficult to determine whether the failure was due to DNA quality or lack of the target *Pseudocercospora* species. Pure cultures of the *Pseudocercospora* spp. were requested from CBS culture collection and these have been received in Dar es Salaam after obtaining the necessary documents from the Tanzanian authorities. They are being kept there. DNA was extracted and sent to Uganda for the optimization. Work to optimize PCR and type all samples for the other species associated with Sigatoka is in progress (Annex 1.7.1[Kimunye]).

Objective 4. Bioassay

The inoculation method for Fusarium wilt was optimized in the past 6 months using Gros Michel plantlets. The effect of inoculum level and inoculation method on banana Fusarium wilt development has been completed. In this experiment, banana plantlets were inoculated using three inoculation methods and different concentrations of the fungus. The inoculation methods included a Foc drenching technique, a Foc-colonized millet seed method, and a combination of dipping of plants without root clipping in a Foc

suspension followed by planting in soils with Foc-colonized millet seed. Plants showed typical external symptoms 3-4 weeks after inoculation (Annex 1.7.1 [Ndayihanzamaso]).

The combined inoculation method at all concentrations caused significantly more disease than other methods (Annex 1.7.1 [Ndayihanzamaso]). The treatment of 2 g of millet seeds per 1 kg of soil was, therefore, selected as the best inoculation method, as it resulted in a RDI that was neither too high, nor too low, to distinguish susceptible from resistant banana varieties/cultivars.

In another study it was shown that Fusarium co-infected with nematodes resulted in a faster infection of Fusarium and earlier and more severe Fusarium symptoms (Annex 1.7.2[Mduma]).

The nematode infection of a single root per sucker with *R. similis* (see WP 3) and *in vitro* plants with *P. goodeyi* (Annex 1.7.2[Mduma]) is working well and was not further optimized.

To develop a weevil bioassay with excised corms, research was initiated to avoid deterioration of corms for at least 30 days. Two media types were evaluated *viz*, 0.4% water agar (w/v) and MS proliferation medium. 0.4% water agar (w/v) was supplemented with different concentrations of filter sterilized Gibberellic acid (GA_3) and BAP. Corms on 0.4% water agar (w/v) supplemented by 8.0 mg/l Gibberellic acid (GA_3) retained the original corm colour. This could indicate that the corm tissues remain alive as would have been the case in a field experiment. Therefore, these preliminary findings have been used on known S and R genotypes to modify the detached corm bioassay for weevil screening based on weight gain of the corm and retention of the corm color.

The detached leaf assay was optimized and protocols for both detached leaf assay and inoculation of small plants were developed (Annex 1.7.1[Kimunye]). Preliminary results reveal that variety response to Sigatoka from the detached leaf assay is similar to the response following field evaluations. More disease streaks were observed on leaf discs inoculated with lower concentrations (0.05 mg/ml) of mycelial fragments which proved more reliable in discriminating between resistant and susceptible varieties, and which was also earlier - 7 days post inoculation (Annex 1.7.1[Kimunye]). There has been a challenge obtaining sufficient quantities of *P. fijiensis* conidia. Currently several media are being tested to optimize spore production. Field trials to evaluate response in adult plants that will be used to validate small plant response to Sigatoka have been established and data collection is in progress.

Young plants of several varieties were inoculated with different concentrations of weighted mycelial suspensions in the screen house to optimize type and level of inoculum and the age at which small plant screening reflects field response. Initial streak symptoms were observed 14 days after inoculation on susceptible varieties. Data collection and monitoring is ongoing. Spore production by *P. fijiensis* has proved challenging however; experiments using different media formulations and incubation conditions to enhance sporulation are thus currently underway to optimize spore production**.** Once fully optimized, the protocol will be validated by inoculating a set of differential cultivars whose response is under evaluation in the field.

A field trial was established in Sendusu to validate the artificial screening protocol. The first field evaluation was conducted six months after planting and thereafter evaluated quarterly. Significant differences in disease severity were observed among genotypes. Disease symptoms also varied from stage 2 in the resistant cultivars to stage 6 in the susceptible genotypes.

Objective 5. Matooke and Mchare (plus hybrids) screening

Mchare banana cultivars were planted late in the project due to their unavailability in Year 1 and complications with varietal nomenclature. Tissue culture plantlets were produced at NARO-Kawanda and the IITA-Arusha station. After multiplication, the plantlets were hardened for 2-3 months and then evaluated under both screen house and field conditions. Mbwazirume (EAHB-AAA) and Sukari Ndiizi (AAB) were included as the resistant and susceptible controls, respectively, at Kawanda, whereas Grande Naine (AAA) and Sukari Ndiizi served as controls at Arusha. The field and screenhouse trials were established in April and May 2017 at Kawanda and Arusha, respectively (Annex 1.3.8).

The millet seed inoculation technique was used for the screenhouse trials. Isolates from infected banana fields at Kawanda and Arusha were used to prepare inoculum in the two countries.

The susceptible control, as well as the Mchare cultivars, developed typical symptoms of Fusarium wilt, which included the yellowing of leaves and brown discoloration of the rhizome. Disease development was slow at both sites, even for the susceptible control. None of the Mbwazirume and Grande Nain plants developed any symptoms (Annex 1.3.8).

Arusha: All Mchare varieties, except Nshonowa, became infected with Foc race 1, ranging from 4 to 21% incidence. Disease severity was significantly lower than for the susceptible control. However, the disease severity of Mchare varieties was also not significantly different from Mbwazirume, the resistant control.

Kawanda: Mchare, Mchare Mlelembo and Nshonowa developed symptoms of Fusarium wilt at incidences of 23, 10 and 33%, respectively. The severity of Mchare and Mchare Mlelembo was low and significantly different from the susceptible control. Nshonowa was most infected, similar to Sukari Ndizi, the susceptible control. Muraru, Kahuti, Kamunyila, Hutishamba and Njuru did not develop Fusarium wilt symptoms.

It was not possible to compare the results of the Mchare varieties in the two countries because their names are different and there is no reference on synonyms. Mchare cultivars with similar names in the two countries showed that Nshonowa was susceptible at Kawanda but not at Arusha. Although the name is the same, their exact identity remains uncertain.

Nevertheless, a tentative ranking of the Mchare cultivars to Fusarium wilt was presented (Annex 1.3.8). Those that developed symptoms but did not differ significantly from the susceptible control were considered susceptible, while infected cultivars that developed symptoms and grouped together with the resistant control varieties were considered as intermediate.

A field screening trial for resistance to banana weevils for the 8 Mchare diploids has been established at Kawanda (NARO) (Annex 1.7.2 [Kemigisa]). At 9 months after planting, 10 weevils in a ratio of 1:1 female to male will be released on each mat. Likewise a weevil screening has begun in Arusha (Annex 1.7.2 [Yusuf Mohamad]).

The number of weevil catches on banana genotypes grown at the banana breeding sites was highest on EAHB (Matooke) genotypes with an average of 4.2 weevils captured per trap, which was lowest from Matooke tetraploid genotypes with an average of 2.5 weevils per trap. The average weevil damage was 1.1% on Mchare genotypes, 22.5% on Matooke genotypes, and 2.8% on Matooke tetraploids (Annex 1.3.3-4-5). In fact most of the Mchare genotypes showed no damage due to the banana weevil with the exception of the genotype "Mchare Laini" (i.e. the Mchare line with the softest fruit after cooking). This implies that either the population of banana weevils in this field is low or Mchare genotypes could be resistant to banana weevil damage.

The screening for nematode resistance will be discussed in WP3 as part of the search for QTLs for nematode resistance.

Evaluation of Sigatoka at regional testing sites was undertaken at least twice for each site and the data analyzed to show the response of NARITAs to natural infection (Annex 1.7.1 [Kimunye]). Samples of diseased leaves were collected at each disease assessment time from evaluated plants; the type of Sigatoka pathogen was detected by PCR to confirm pathogen identity and isolations made to see if there is a change in pathotypes. This remains an on-going activity.

EAHB hybrids planted at Sendusu and Kawanda have been evaluated four times (evaluations conducted quarterly) and at each evaluation time, samples are collected to monitor pathogen identity and pathotype variation. There was no significant difference in severity of Sigatoka among the hybrids (Annex 1.7.1 [Kimunye]). Preliminary data indicates that Sigatoka severity in the NARITAs is lower than the local EAHB (Matooke) checks. However, the disease was more severe on mature plants. Environmental data will be incorporated in the analysis to determine the role of the environment on disease severity.

There was no significant difference in cultivar response to Sigatoka over the evaluation period among Mchare genotypes but significant differences were observed between the evaluation times. Significant differences in severity indices were observed on plants at different developmental stages. The difference is probably due to weather conditions and/or management practices like de-trashing that removes old and diseased leaves. Environmental aspects will be incorporated in the analysis.

Primary outcomes 3: WP 3 on Leveraging Genetics (Genetics of resistance to *Fusarium oxysporum* f.sp. *cubense* (Foc), burrowing nematode (*Radopholus similis*) and weevil determined in banana facilitating development of molecular markers for banana breeding; and a SNP-based genomic model for breeding for yield and agronomic traits in Matooke and Mchare developed.

Scope and approach: Improved breeding efficiency through molecular-based genetic studies for increased understanding of underlying genetics and development of DNA marker-based early selection.

The goal of this work package is to develop molecular markers and QTLs for 3 pests and diseases and support breeding by following up true-to-typeness of genotypes.

Therefore the objectives are (now reworded from the submitted project document):

1. Phenotyping the mapping populations and the training population;
2. Developing a genomic selection model;
3. Molecular characterization of the mapping populations and other accessions using SSR markers;
4. Leaf archiving.

Objective 1. Phenotyping for disease and pest resistance/susceptibility trait

8 mapping populations are available (Annex 1.4.1). We had previously reported a higher number of mapping populations but removed some because of mistakes. 3 mapping populations are at NARO-Kawanda, 3 at IITA-Sendusu and 2 at IITA-Arusha. They were designed to study segregration for Fusarium, nematodes, weevils and Sigatoka plus other traits such as bunch orientation, parthenocarpy and plant size. One population was developed by UQ and the others by NARO and IITA. The number of plants per mapping population ranges from 82 to 296.

Phenotyping made substantial progress for Fusarium, nematodes and weevils (Annex 1.4.2-3-4).

Segregation for Foc R1 was confirmed in Monyet x Kokopo (Annex 1.7.1 [Arinaitwe]), Calcutta 4 x Calcutta 4 and Paliama x Borneo. Intensive phenotyping is going on in Monyet x Kokopo and Paliama x Borneo (Annex 1.7.1 [Mpina]). 74% and 34% of Monyet x Kokopo and Paliama x Borneo populations have been phenotyped respectively. Phenotyping for Foc SR4 and TR4 continues in Australia (UQ). A number of genotypes including those from the Malaccensis x Malaccensis population await inoculation with TR4. The Foc SR4 QTL was fine-mapped to 5 molecular markers closely linked to the QTL, covering a genetic distance of 6.7 cM. This region of 157 kb of sequence appears to harbour 27 predicted gene models which makes it a region of relatively high gene density. To look at the transcript profiles of the candidate genes, early infection process was examined using a subtropical Foc Race 4 strain (VCG0120) transformed with a green fluorescent protein (GFP). Gene expression levels indicated 2 candidate genes that were significantly up-regulated in the roots of the resistant Malaccensis at 1-3 days post inoculation, in comparison with the untreated controls and the treated susceptible Malaccensis. This work is ongoing with the expression of other genes to be analyzed next. Work is on-going to examine the presence and absence of genes in our Malaccensis that could have been missed in the DH Pahang genome, using the Oxford Nanopore sequencing and Illumina HiSeq4000 platforms to sequence the parents.

Two populations are being screened for nematode resistance: Kasaska x Borneo and Calcutta 4 x Zebrina GF (Annex 1.7.2 [Habineza]). 89% of the Kasaska x Borneo population has been phenotyped. For weevil resistance, phenotypic data are available for half of the population (124 out of 253 genotypes). The population Monyet x Kokopo is also being phenotyped for this trait (Annex 1.7.2 [Mwanje]), and 23% of the population was completed in the last five months, and more experiments are running.

Objective 2. Developing a genomic selection model (Annex 1.7.1 [Nyinye], Annex 5)

Phenotypic data have been collected in the training population made of 307 in three fields. Two fields are at Sendusu, under contrasting management: one with high input (with manure, yearly application of NPK and mulching); one under low input (manure applied at planting only, no NPK, no mulching). The third field is located in Mbarara, under high input management. Phenotypic data are collected on yield

and other agronomic traits at flowering and harvest. The data are to supplement the training population data, and potentially to serve in validation of the predictive models. Two crop cycles are almost complete and the third cycle is evaluated (Annex 1.4.2).

A PhD thesis was submitted and is under review before a defense date can be set up (Annex 5).

The prediction models used were Bayesian ridge regression (BRR), Bayesian LASSO (BL), BayesA, BayesB, BayesC and reproducing kernel Hilbert space (RKHS) to assess 15 traits. The aim was to investigate the potential of genomic selection (GS) as a method of selection that could benefit breeding through increased genetic gain per unit time and cost. Trait variation, the correlation between traits and genetic diversity in the training population were analyzed as an essential first step in the development and selection of suitable genomic prediction models for banana traits. Clustering based on SSR markers revealed that the training population was genetically diverse. Models that account for additive genetic effects provided better predictions with 12 out of 15 traits. The performance of BayesB model was superior to other models, particularly for fruit filling traits (fruit length, fruit circumference, pulp diameter) with a prediction ability of 65-72%; and for bunch weight (62-64%). The prediction models will be fine-tuned using the phenotypic data from Mbarara and data coming in from the third cycles of the 3 sites. Validation was partly possible using the phenotypic data currently collected from 200 genotypes from EET (Annex 1.4.2).

Fruit filling is an important trait in banana breeding, as it is the basis for rejecting over 80% of the EET material. Following the observations made from genomic predictions, the data were analyzed for fruit-filling using the GWAS method, which identified a promising region on chromosome 3. Specific PCR based markers are being developed for this region as an alternative to genomic prediction for in-house analysis of fruit filling.

Objective 3. Molecular characterization using SSR markers.

A total of 526 accessions were genotyped during this period, representing several mapping populations, including the parents of the progeny and other material for which the genetic diversity was analyzed by SSR markers (Annex 1.4.5) and placed on the dendrogram together with the ITC core collection genotyped at IEB (Annex 1.4.6). Mapping populations were genotyped to check their true-to-typeness, relative to the intended crosses and in some cases showed discrepancies. Other accessions were genotyped to check their diversity, compared to the core ITC set genotyped at IEB. Noteworthy is the fact that the Mchare/Muraru/Mlali accessions from Arusha/Tanzania and Kenya are all grouping together.

A QTL for Foc SR4 has been identified on chromosome 3 by the team at UQ. The QTL has been converted into a PCR-based marker and confirmed in known Malaccensis resistant genotypes. DNA for 18 genotypes was sent to UQ for genotyping for this specific QTL. These included 7 diploid parents used at Sendusu, which are Malaccensis or Malaccensis-derived (having a Malaccensis in their pedigree) and 11 accessions used in WP 2 screening for Foc R1. These were run together with 2 known controls (resistant and susceptible) (Annex 1.4.7).

Among the Malaccensis and Malaccensis-derived diploid parents sent from the breeding program, only SH3217 has the Foc SR4 QTL in a homozygous resistant state. TMB2x7197-2, 5601S-1, SH3362 and Malaccensis-250 are heterozygous and the rest of the tested genotypes were homozygous susceptible. SH3362 and Malaccensis 250 were found to be resistant. TMB2x8075-7 carries the resistant allele. SH3217 is the female parent of SH3362, and in this cross the source of resistance seems to have carried through. Most of the NARITAs (derived hybrids, 3x ploidy) tend to have either SH3362 or SH3217 as the male parent. This increases the chance that some lines are resistant against Foc TR4. The PYT and AYT selections have a significant portion of lines that were derived from 5610S-1 as either a male or female parent or Malaccensis (Malaccensis 250) as the male parent. SH3362 and SH3217 were also the male parents of half of the genotypes in advanced stages of selection. Resistance against Foc TR4 should be expected from some of these genotypes. The team at UQ has shared with IITA the primer and cutting enzyme information for this marker. Further screening will be conducted in Arusha and Sendusu.

Objective 4. Leaf archiving (Annex 1.4.8)

IITA and NARO have begun the leaf archiving of all genotypes being phenotyped under WP1-4. Samples have been collected at Sendusu, Kawanda and Arusha. The leaf samples are kept at -80°C while waiting to be freeze-dried. Leaf samples of 2688 genotypes were archived. **Error! Reference source not found.**The sampled material constitutes about 80% of the material to be archived at Sendusu, 25% of the material in Kawanda and 50% of the material in Arusha. Leaf sampling will continue in the first half of Year 4 in order that all the genotypes under phenotyping will be archived.

Primary outcomes 4-5-6-7: WP 4 on Regional Testing and end-user evaluation

Scope and approach: Empowering End-user Evaluation: System for better tailoring breeding products and increasing adoption of new cultivars through end-user feedback systems and participatory evaluation of improved banana germplasm.

The goal of this work package is geared towards improving the acceptance rate of high yielding NARITA hybrids by local farmers.

Therefore the objectives are (now reworded from the submitted project document):

1. Identify the best hybrids according to consumer evaluations;
2. Conduct sensory evaluations by consumers: provide qualitative feedback to breeders on taste and other organoleptic features, as well as processing potential;
3. Determine farmer preferred traits: towards modifying the breeding strategy to maximize acceptability of hybrids;
4. Establish a baseline study: to facilitate future release of hybrids;
5. Conduct multi-location trials in a range of target end-user environments: to select the best hybrids per environment, better understand G x E, collate information for rapid release;
6. To evaluate at least 20 of the 27 NARITA hybrids.

This is organized in four primary outcomes

Primary outcome 4. Breeders have a better understanding of traits of importance to end users and use this to orientate breeding strategies and early selection processes.

Primary outcome 5. Simplified, standardized protocol and tools for trial design and implementation, data collection and sharing implemented by all partners allowing meta-analyses across sites.

Primary outcome 6. Farmers participating in selection of new hybrids, with feedback driving changes to strategy and selection processes of breeding programs to improve tailoring of future improved hybrid

Primary outcome 7. Farmers across Uganda and Tanzania and beyond growing their preferred NARITA cultivars, alongside local cultivars.

Primary outcome 4. This serves objectives 1-4.

A first draft on banana products was prepared from farmers' group discussion (FGD). This results in a wealth of information that now needs detailed interpretation. So far 33 products were identified (Annex 1.5.1, table 1). These products were linked to the 4 banana types (cooking, dessert, roasting and beverage) in 5 testing sites (Annex 1.5.1, table 2). Moreover the varietal performance to deliver either a good or bad product was identified (Annex 1.5.1, table 3-4). This shows that criteria for a good product depends on the location and that the value of a variety depends on the desired product. It became clear that an earmarked trait can also be linked to the status both before or after processing. Hence giving the huge number of variables (products x traits before and after processing x location x variety) breeding for the end-user is therefore very complex and would benefit from biochemical quantification of the 2 main products (Matooke and Mchare as listed in Annex 1.5.1, table 2) to develop a high throughput screening method. Moreover much greater emphasis needs to be placed on the testing of hybrids for consumption in the different locations.

Given the numerous varieties, there was a need to properly identify and name each variety and thus to develop a banana cultivar name and synonym dataset. This dataset has been sent to a wider array of colleagues for final checking and will soon become available.

Work on the baseline intra-household survey datasets is close to finalization and will be available soon. This involved the collation of seven different datasets of the five districts, and the cleaning, sorting, calculation of indexes, etc. The data analysis pipeline was set up, and the first draft with descriptive statistics and plots for the technical report are being generated.

A systematic literature review to assess the scope of published and grey literature on gender-differentiated banana trait preferences across the value chain was performed. Farmers and farming households prefer a large range of traits in their role as producers, processors, marketers and consumers of banana. A summary of their preferred attributes was grouped into five main categories for each of the banana types (Annex 1.5.1, table 5): physical agronomy (21), size and shape attributes (15), sensory/organoleptic (15), processing and product related (18), socio-economic descriptors (8), and others (5). It is striking that cooking bananas by far fulfill the highest number of these characteristics, followed by plantain. However there has been a bias in the number of studies linked to specific traits and banana groups, which necessitates caution in interpretation, while many studies also lack proper details. The fact that cooking bananas received a larger set of traits, might simply be due to the fact that cooking bananas are more appreciated than other banana types.

The review will provide background knowledge that will be necessary for future research activities that include analysis and publication of the baseline data from the household surveys and FDGs, and protocols for the sensory evaluations and preference ranking of the NARITAs during farmer field days.

Primary outcome 5. This serves objectives 5-6.

The banana trait ontology has now been uploaded to the Crop Ontology website (http://www.cropontology.org/ontology/CO_325/Banana).

Following the data collection protocol workshop held at Kawanda in September 2016, and the final labelling of the plants in each site with the QR codes, the data collectors took practice data from October-December 2016, and final issues in the mobile data collection system were ironed out. A server was established in January 2017 to host the mobile data from the five field sites: https://bio.smap.com.au/.

Discussions were initiated to create a dashboard within the server where the data is received from the field trial data collectors that provides real-time monitoring of recording at the field trials.

The field trial layout information for the five WP4 field trials was uploaded into MusaBase.

A protocol design and preparations for the sensory evaluations of the NARITAs is currently underway. The evaluations will assess organoleptic characteristics related to the appearance and consumption of food (and beverage) products to determine potential end user acceptability of the NARITAs.

Primary outcome 6. This serves objectives 5-6.

Plants have reached harvest stage in most sites. Data collection is ongoing and sent monthly to the server, which are checked and feedback provided to the site managers and data collectors.

An issue of mislabeling of plants in all sites was identified. The team is checking the status of all plants, to understand the source and the extent of the issue and are correcting labels as necessary.

Two tissue-culture companies were contacted for the multiplication of planting materials for the on-farm trials. Given that the NARITAs are not yet available from the ITC, it was agreed to order starting materials from IITA-Nigeria. The set of NARITAs for the on-farm trials in Tanzania has already arrived at Kilimorgano, Tanzania and shipment to BioCrops, Uganda is expected soon.

Primary outcome 7. **This serves objectives 4-6.**

The Musapedia page on the NARITA hybrids and the individual NARITA factsheets on the ProMusa website received ~3500 page views, with visitors coming from over 70 different countries (top ranked including Uganda and Tanzania).

A review was made of different documents outlining the varietal release guidelines in Uganda and Tanzania (Annex 1.5.2). The document was circulated and discussed between partners in both countries. Discussions to fully understand the procedures and data/experimental requirements for varietal release will be continued in year 4.

In collaboration with RTB cluster CC2.1 (Quality seeds and access to improved varieties), a draft workplan was developed for a gendered analysis of the seed systems in target regions for adoption of the NARITAs (Annex 1.5.3). The aim is to better understand the existing mechanisms and key actors for seed exchange, in order to identify opportunities and constraints for the introduction of new varieties in the banana production systems in the target regions, and the critical factors for success. We seek to better understand where the target end-users source their materials for new banana plantings, how they access information about quality planting materials of diverse varieties, and how they make decisions about which varieties and which materials to use for their new plantings. Emerging knowledge from this study will be used to inform upcoming activities on the dissemination of selected NARITAs in the target regions.

Within the new project proposal RTB FOODS, the team will conduct community-level evaluation of processing and sensory descriptors for two key banana-based products (Matooke in Uganda and Mchare in Tanzania), and conduct consumer taste tests with rural and urban communities for these key products.

Primary outcomes 8: WP 5 Create a banana breeding database

Scope and Approach: Harnessing Data: Driving improved efficiency of breeding systems and enhanced synergy in national, regional and global partnership through an open-source database and tool box for banana breeders and researchers

The goal of this work package is to create a banana breeding database to improve the efficiency in banana breeding.

The objective is to provide project partners and Musa researchers and breeders a virtual hub for information exchange, R&D collaboration and enhanced adoption of new hybrids. Major progress was achieved in:

1. Development of a banana cross tracking tool: A system for tracking seed, monitoring progress and reporting results in banana breeding.

   IITA Nairobi with support from IITA Arusha and BTI developed a new crossing tool (a standalone application using a handheld device) that will become interconnected to MusaBase. The tool framework was designed and implemented in MusaBase where a crossing wish list (https://musabase.org/breeders/crosses/) can be generated and sent to the new cross app.

   The app itself is currently being developed in Open Data Kit (ODK) environment using ONA server (https://ona.io/cassavaseedtracking/33345) including some analytics in R by IITA Nairobi (https://github.com/mkaranja/banana_pipeline). Full details of the tool are provided in Annex 1.6.1.
   Margaret Karanja of IITA Nairobi visited BTI in July for a month to familiarize herself with MusaBase and collaborated with the development team on the cross app.
2. Updating of the crop ontology tool. The trait ontology established in 2016, covering breeding (WP 1-3) and supplemental variables (WP 4) was updated based on breeder's feedback (https://musabase-test.sgn.cornell.edu/tools/onto/). An additional tool was developed to allow the building of own traits, including a time variable directly from the web interface (https://musabase-test.sgn.cornell.edu/tools/compose).
3. Training of three data base managers (NARO, IITA Sendusu, IITA Arusha) at BTI to accelerate the use of MusaBase by banana breeders but also to provide first-hand experience to BTI to adapt MusaBase to banana breeder's needs (Annex 1.6.2). The database managers received training on:
   - features of the MusaBase Sol genomics Platform;
   - curating and managing accessions (adding new accessions, editing, etc.);
   - developing database formats compatible with MusaBase including trials, traits, field plans/layouts;
   - preparing new and existing trials for uploading into MusaBase;
   - the relationship and interphase between the field book and MusaBase;
   - barcoding;
   - setting up crosses using the wish list feature.

Upon return, there was a boost in uploading data and field maps onto MusaBase.
The focus now will be to begin working in the field with tablets and also strengthen the link to WP 2 on pests and diseases.