Novel Approaches to the Improvement of Banana Production in Eastern Africa - the application of biotechnological methodologies

NARO-Bioversity Banana Improvement Project

Technical Report, 1 July 2019 to 30 June 2020

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1. Executive Summary

The project's overall objective is to build capacity for the development and deployment of biotechnological tools and products critical for the transformation of Uganda's agricultural economy. The focus is to improve resistance to pests (weevils, nematodes) and diseases (bacterial wilt, black Sigatoka and Fusarium wilt) in the East African highland bananas using novel biotechnological approaches. Despite a delay in release of funding, the team continued some of the Project activities. This report therefore builds on the end of Phase IV project report, but specific emphasis on the research outputs for the period 1 July 2019 to 30th June 2020 whose key activities included;

a) Maintenance in screen and green house facilities of transgenic lines selected for resistance to nematodes and weevils
b) Establishment of regulatory facilities in the confined field trial at NARL, Kawanda
c) Establishing and maintaining nematode and weevil inoculum to inoculate CFT test plants
d) Management and conservation of the regional Germplasm collection
e) Ph.D studentships
f) Construction of the laboratory at NARL, Kawanda

Prior to establishment of transgenic banana lines in the Confined Field trial (CFT), potted plants are established and maintained under screen /glass house conditions. Therefore, transgenic lines of 'Nakitembe' and 'Gonja Nakatansense' for weevil and nematode resistance were and are still maintained in screenhouse and glasshouse awaiting planting in the CFT. Out of a total of 161 transgenic lines under maintenance in the screenhouse, 41 plantain Cultivar Gonja lines and 99 Nakitembe lines have been selected and are ready for planting in the CFT. Additionally, transgenic lines confirmed for planting in the CFT were re-initiated *in vitro* to provide back-up materials for future use. In accordance with guidelines and Standard Operating Procedures of field trials of genetically engineered plants, the CFT site already approved by the National Biosafety Committee (NBC) was kept under 24/7 security surveillance. In preparation for inoculation of transgenic lines, weevil and nematode inoculum was maintained.

Activities geared to management and conservation of the Regional germplasm collection continued during the project reporting period. Two Ph.D students from phase three of biotech project continue making very good progress despite Covid-19 interference.

2 Activity 1: Maintenance in screen and green house facilities of transgenic lines selected for resistance to nematodes and weevils

As reported in end of Phase III report, this work explores the application of insecticidal and nematidical properties of *Bacillus thuringiensis* (Bt) crystalline (cry) proteins and plant proteinase inhibitors (cystatins) in the control of weevils and nematodes which are major pests of bananas in Uganda. As such, transgenic banana lines with Bt *Cry6A*, *Carica papaya* cystatin (*CpCYSΔ89*) and a stack of the two genes were developed and evaluated under screenhouse conditions for resistance to weevils and nematodes. During this period (2019 – 2020), the selected lines have been maintained in pots under screen and glasshouse conditions awaiting planting in the approved CFT site to be further evaluated under field conditions. Additionally, all lines confirmed for planting in the CFT are being maintained with approximately 10 plants under tissue culture conditions as back up for future use. As a result of the delay in release of research funds, all the selected transgenic lines and their non-transgenic controls (Fig. 2.1; Tables 2.1 – 2.9) were maintained in pots under screen house conditions prior to planting in CFT. Therefore, the project research team maintained the screenhouse plants which involved watering, application of foliar fertilisers, fumigation and detrashing. Additionally, overgrown potted plants were routinely cut back which would otherwise create an overgrowth in the screenhouse facilities.

Figure 3:1: Ready to plant transgenic lines (A; cut back, B; re-established) in pots being maintained under screenhouse and glasshouse conditions.

Table 3:1: Controls of Nakitembe (AAA), Gonja (AAB) and Yangambi KM5

Cultivar	Phenotype	Copies targeted	Copies in pots
Nakitembe	Non-transgenic	30	90
KM5	Non-transgenic	30	37
Nakitembe	GUS-transgenic	30	58

Table 3:2: Selected transgenic lines of Gonja (AAB) for resistance to nematodes *Radopholus similis*

Transgene	Line	Copies targeted	Copies in pots
CpCYSΔ89	16	20	12
CpCYSΔ89	33	20	19
CpCYSΔ89	35	20	29
CpCYSΔ89	37	20	60
CpCYSΔ89	50	20	28
CpCYSΔ89	139	20	48
Cry6A	45	20	20
Cry6A	61	20	27
Cry6A	96	20	12
CpCYSΔ89 + Cry6A	5	20	21
CpCYSΔ89 + Cry6A	17	20	10
CpCYSΔ89 + Cry6A	20	20	20
CpCYSΔ89 + Cry6A	23	20	36
CpCYSΔ89 + Cry6A	26	20	15
CpCYSΔ89 + Cry6A	27	20	10
CpCYSΔ89 + Cry6A	48	20	20
CpCYSΔ89 + Cry6A	57	20	31
CpCYSΔ89 + Cry6A	96	20	29

CpCYSΔ89 + Cry6A	115	20	38
CpCYSΔ89 + Cry6A	116	20	21
CpCYSΔ89 + Cry6A	134	20	17
21 lines			

Table 3:3: Selected transgenic lines of Nakitembe (AAA) with *CpCYSΔ89* for resistance to nematodes *Radopholus similis*

Transgene	Line	Copies targeted	Copies in pots
CpCYSΔ89	3	20	20
CpCYSΔ89	5	20	28
CpCYSΔ89	8	20	28
CpCYSΔ89	27	20	22
CpCYSΔ89	28	20	21
CpCYSΔ89	34	20	27
CpCYSΔ89	35	20	11
CpCYSΔ89	52	20	16
CpCYSΔ89	55	20	15
CpCYSΔ89	60	20	15
CpCYSΔ89	86	20	31
CpCYSΔ89	91	20	60
CpCYSΔ89	97	20	23
CpCYSΔ89	109	20	31
CpCYSΔ89	115	20	23
CpCYSΔ89	120	20	31
CpCYSΔ89	123	20	21
CpCYSΔ89	144	20	16
CpCYSΔ89	154	20	31
CpCYSΔ89	164	20	17
CpCYSΔ89	171	20	29
CpCYSΔ89	177	20	29
CpCYSΔ89	178	20	26
CpCYSΔ89	179	20	32
24 lines			

Table 3:4: Selected transgenic lines of Nakitembe (AAA) with *Cry6A* for resistance to nematodes *Radopholus similis*

Transgene	Line	Copies targeted	Copies in pots
Cry6A	10	20	23
Cry6A	15	20	28
Cry6A	18	20	14

Cry6A	38	20	31
Cry6A	39	20	48
Cry6A	49	20	21
Cry6A	90	20	22
Cry6A	97	20	50
Cry6A	116	20	28
Cry6A	140	20	31
Cry6A	161	20	17
Cry6A	176	20	18
Cry6A	189	20	18
Cry6A	191	20	22
Cry6A	202	20	23
Cry6A	242	20	33
Cry6A	248	20	34
17 lines			

Table 3.5: Selected transgenic lines of Nakitembe (AAA) with stacked (*CpCYSΔ89* and *Cry6A*) for resistance to nematodes *Radopholus similis*

Transgene	**Line**	**Copies targeted**	**Copies potted**
CpCYSΔ89 + Cry6A	18	20	35
CpCYSΔ89 + Cry6A	31	20	15
CpCYSΔ89 + Cry6A	35	20	32
CpCYSΔ89 + Cry6A	36	20	26
CpCYSΔ89 + Cry6A	38	20	30
CpCYSΔ89 + Cry6A	44	20	27
CpCYSΔ89 + Cry6A	50	20	25
CpCYSΔ89 + Cry6A	51	20	39
CpCYSΔ89 + Cry6A	71	20	34
CpCYSΔ89 + Cry6A	105	20	40
CpCYSΔ89 + Cry6A	120	20	23
CpCYSΔ89 + Cry6A	127	20	30
CpCYSΔ89 + Cry6A	131	20	42
CpCYSΔ89 + Cry6A	142	20	19
CpCYSΔ89 + Cry6A	152	20	36
CpCYSΔ89 + Cry6A	153	20	29
16 lines			

Table 3.6: Selected transgenic lines of Gonja (AAB) for resistance to banana weevil *Cosmopolites sordidus*

Transgene	Line	Copies targeted	Copies potted
CpCYSΔ89	12	20	17
CpCYSΔ89	39	20	32
CpCYSΔ89	44	20	30
CpCYSΔ89	48	20	17
CpCYSΔ89	50	20	20
CpCYSΔ89	56	20	21
CpCYSΔ89	72	20	26
CpCYSΔ89	118	20	11
CpCYSΔ89	137	20	29
CpCYSΔ89	138	20	12
CpCYSΔ89	144	20	13
CpCYSΔ89	179	20	36
CpCYSΔ89	189	20	19
Cry6A	74	20	14
Cry6A	90	20	20
CpCYSΔ89 + Cry6A	14	20	44
CpCYSΔ89 + Cry6A	24	20	23
CpCYSΔ89 + Cry6A	101	20	22
CpCYSΔ89 + Cry6A	118	20	13
CpCYSΔ89 + Cry6A	264	20	9
	20 lines		

Table 3.7: Selected transgenic lines of Nakitembe (AAA) with *Cry6A* for resistance to banana weevil *Cosmopolites sordidus*

Transgene	Line	Copies targeted	Copies potted
Cry6A	43	20	16
Cry6A	46	20	9
Cry6A	51	20	18
Cry6A	53	20	20
Cry6A	63	20	23
Cry6A	70	20	19
Cry6A	90	20	19
Cry6A	96	20	17
Cry6A	112	20	18
Cry6A	117	20	15
Cry6A	143	20	47

Cry6A	145	20	45
Cry6A	149	20	23
Cry6A	151	20	13
Cry6A	158	20	21
Cry6A	182	20	25
Cry6A	187	20	24
Cry6A	189	20	47
Cry6A	194	20	25
Cry6A	197	20	20
Cry6A	212	20	16
Cry6A	231	20	29
Cry6A	242	20	23
Cry6A	256	20	18
Cry6A	270	20	11
25 lines			

Table 3.8: Selected transgenic lines of Nakitembe (AAA) with *CpCYSΔ89,* and stacked (*CpCYSΔ89* and *Cry6A*) for resistance to banana weevil *Cosmopolites sordidus*

Transgene	**Line**	**Copies targeted**	**Copies potted**
CpCYSΔ89	50	20	21
CpCYSΔ89	58	20	12
CpCYSΔ89	90	20	26
CpCYSΔ89	168	20	19
CpCYSΔ89	288	20	17
CpCYSΔ89 + Cry6A	28	20	23
CpCYSΔ89 + Cry6A	52	20	41
CpCYSΔ89 + Cry6A	107	20	39
CpCYSΔ89 + Cry6A	152	20	20
CpCYSΔ89 + Cry6A	157	20	24
CpCYSΔ89 + Cry6A	159	20	13
CpCYSΔ89 + Cry6A	181	20	18
CpCYSΔ89 + Cry6A	212	20	33
CpCYSΔ89 + Cry6A	216	20	22
CpCYSΔ89 + Cry6A	220	20	27
15 lines			

Table 3.9: Summary of selected transgenic lines of Nakitembe (AAA) and Gonja (AAB) cultivars

Transgene	Gonja With resistance to:		Nakitembe With resistance to:	
	Nematodes	**Weevils**	**Nematodes**	**Weevils**
Cry6A	3	2	17	25
CpCYSΔ89	6	13	24	5
CpCYSΔ89 + Cry6A	12	5	16	10
Total number of lines	21	20	57	40

3 Activity 2: Establishment of confined field trial facilities at NARL, Kawanda

3.1 Construction of the CFT facilities

The proposed site for the CFT was approved by the NBC and under 24/7 security surveillance in accordance with the Standard Operating Procedures (SOPs) and National Guidelines for Field Trials of Genetically Engineered Crops by Uganda National Council for Science and Technology (UNCST). On June 16th, 2018, the Project was granted permission to construct and plant the selected transgenic lines under field conditions in the CFT under Application Reference Number: NBC/01/2018 with a UNCST Decision Number 1/2018. The required construction to meet regulatory compliance partially started with fencing off the site (previously ploughed) with chain-link and a guard house. The approved site is divided into two experimental fields, testing bananas for resistance to nematodes and weevils respectively (Fig. 2.2).

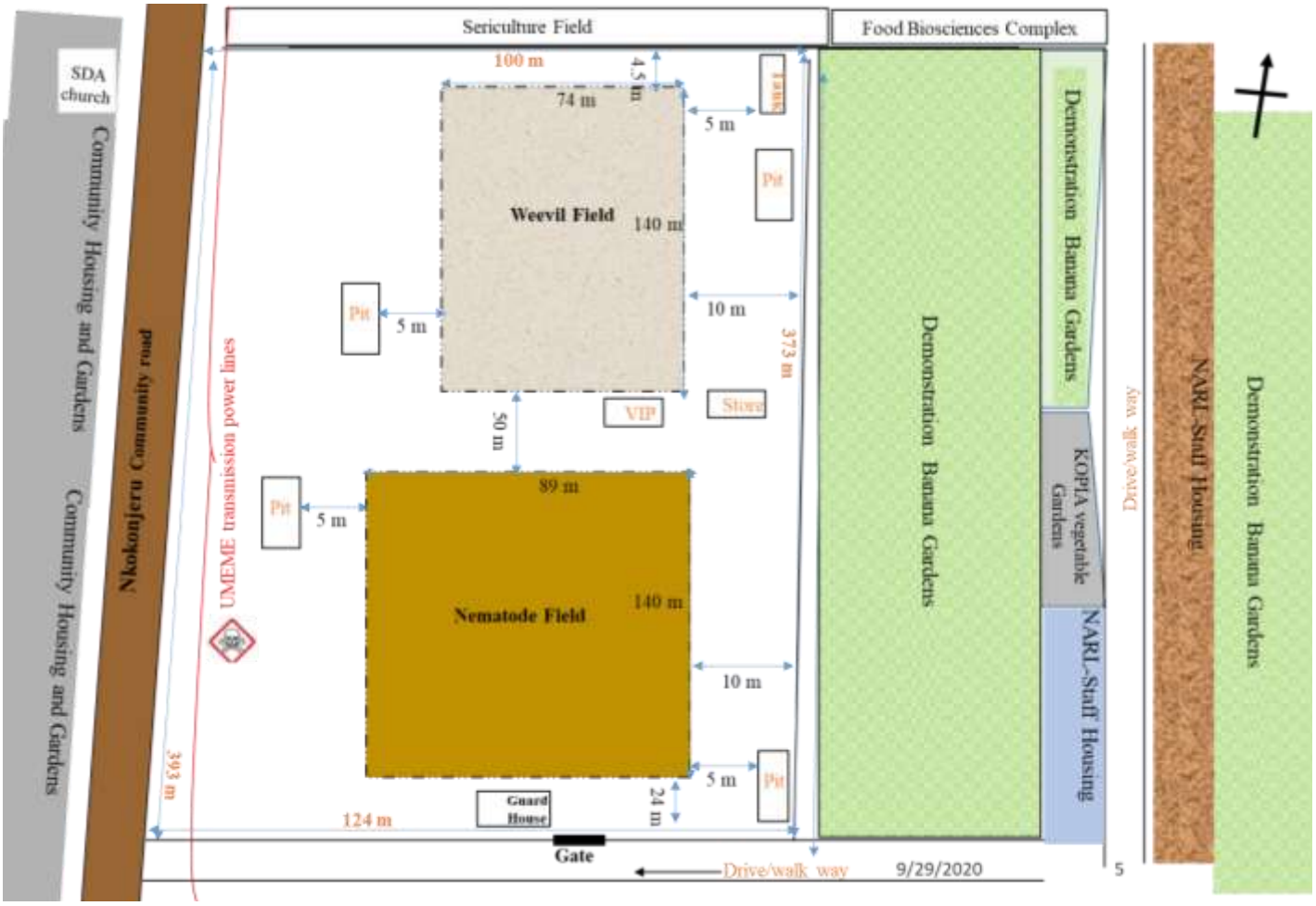

Figure 3.2: The CFT site with the layout of regulatory facilities, water tank and the two fields.

Since there was a delay in commencement of the current phase (Phase IV) of this project, the approved CFT site grew bushy which necessitated slashing of the over-grown shrubs and grass prior to construction of the required facilities. Therefore, the CFT site was cleared of the bush and the dried grass collected and heaped safely to provide mulch for the soon to be established plants in the field (Fig. 2.3). For the confined site to host transgenic plants, regulations required other in-site facilities such as incineration pits, storage space and pit latrines. Therefore, a water supply system including a 10,000 litre reservoir connected to a mains 800 metres away will supply water to the in - CFT site plants via a network of 6 water taps upstream of the tank. Construction of the water line and its storage facility in addition to incineration pits, storage space and pit latrines is completed (Fig. 2.4).

Figure 3.3: Clearing of the CFTS site of overgrown bush (A) and cleared field (B).

Figure 3.4: Construction of the regulatory facilities and water supply system to the CFT site.

3.2 Demarcation and marking of the fields in the CFT site

In preparation for planting, the CFT site hosts two fields, one for nematode (140 metres by 89 metres) and another for weevils (140 metres by 74 metres) whose respective layouts and planting field maps have been developed (Fig. 2.2). The nematode field is divided into three blocks, each with 82 banana lines where each line will be planted with four plants within a square plot. Additionally, a 16 metre long block will host the 82 lines but with two plants each within a plot. Plants in the 28 - 30 m blocks will be inoculated with nematodes while the 16 m block will remain un-inoculated. Plant spacing in both fields is 2 X 3 metres between rows and columns respectively (Fig. 2.5).

The weevil field is divided into three blocks, each with 63 banana lines where each line will be planted with four plants within a square plot. Additionally, a 16 metre long block hosting the 63 lines but with two plants each within a plot. Plants in the 28 - 30 m blocks will be inoculated with nematodes while the 16 m block will remain un-inoculated (Fig. 2.5).

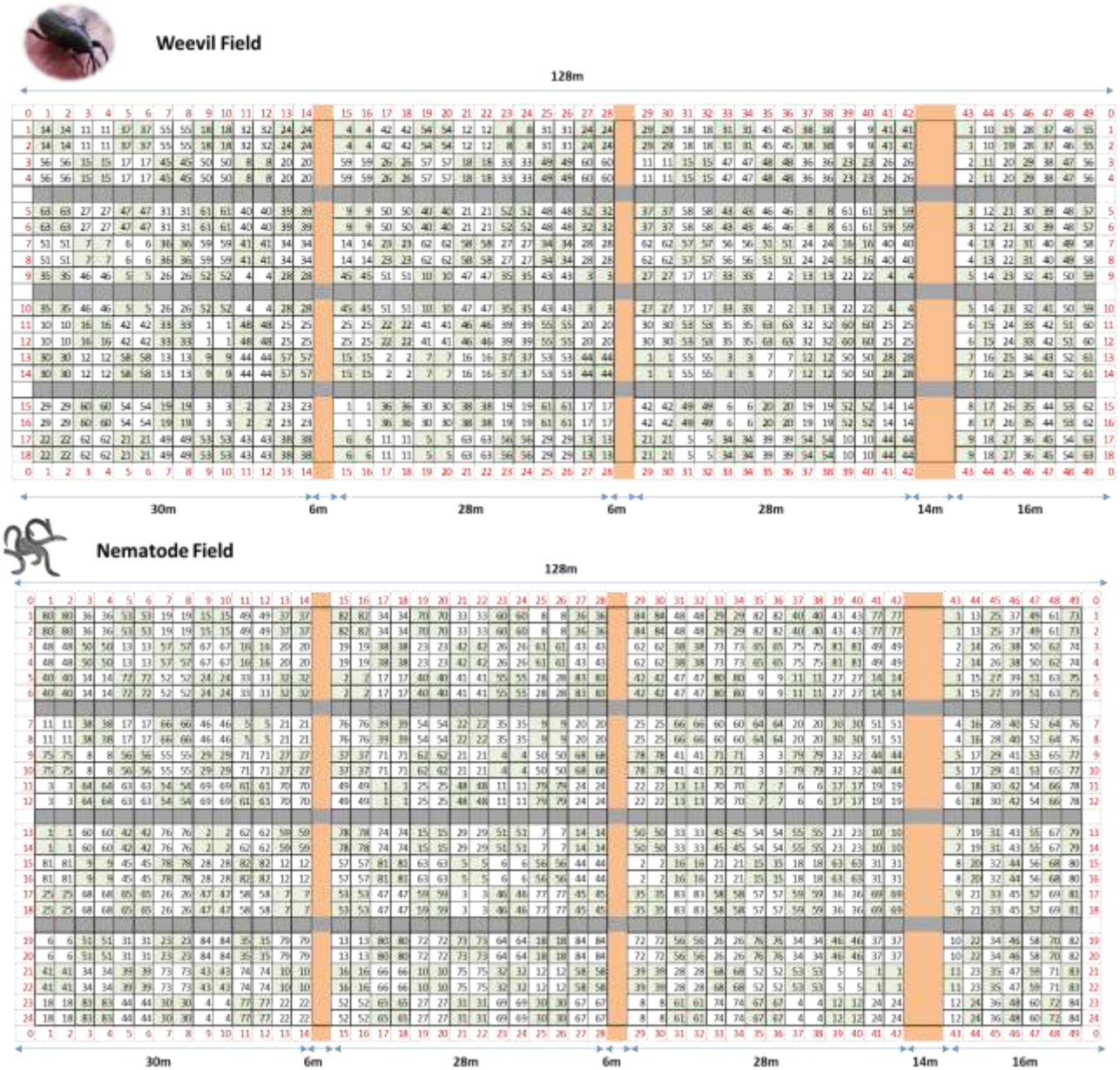

Figure 3.5: The layout of the planting map of the respective two fields in the CFT site.

3.4 Digging planting holes, designing and printing of regulatory signage and in-field plant labels

In preparation for planting, digging of 2,294 holes in the two experimental fields (nematode trial; 1,286 and weevil trial; 1,008) has been procured and works have commenced with digging of nematode field complete. Once digging of all planting holes is completed, cow-dung based manure will be incorporated in the soil around the plants. To facilitate this, 40 trips of manure have been procured for delivery to the CFT site.

Figure 3.6: Digging of planting holes for the nematode inoculated field is completed.

As a regulatory requirement, signage (Fig. 2.7) at the CFT site has been designed and its procurement process completed and being printed to be erected at the entrance of the CFT site before pre-planting inspection by the NBC. Additionally, all test plants in the field will be labelled for clear identification in accordance with the stewardship SOPs. This will be achieved by marking each plant with an in-field label. Both fields require 2,030 labels (nematode trial; 1,148 and weevil trial; 882) which are being printed (Fig. 2.8) and expected to be placed in-field before planting.

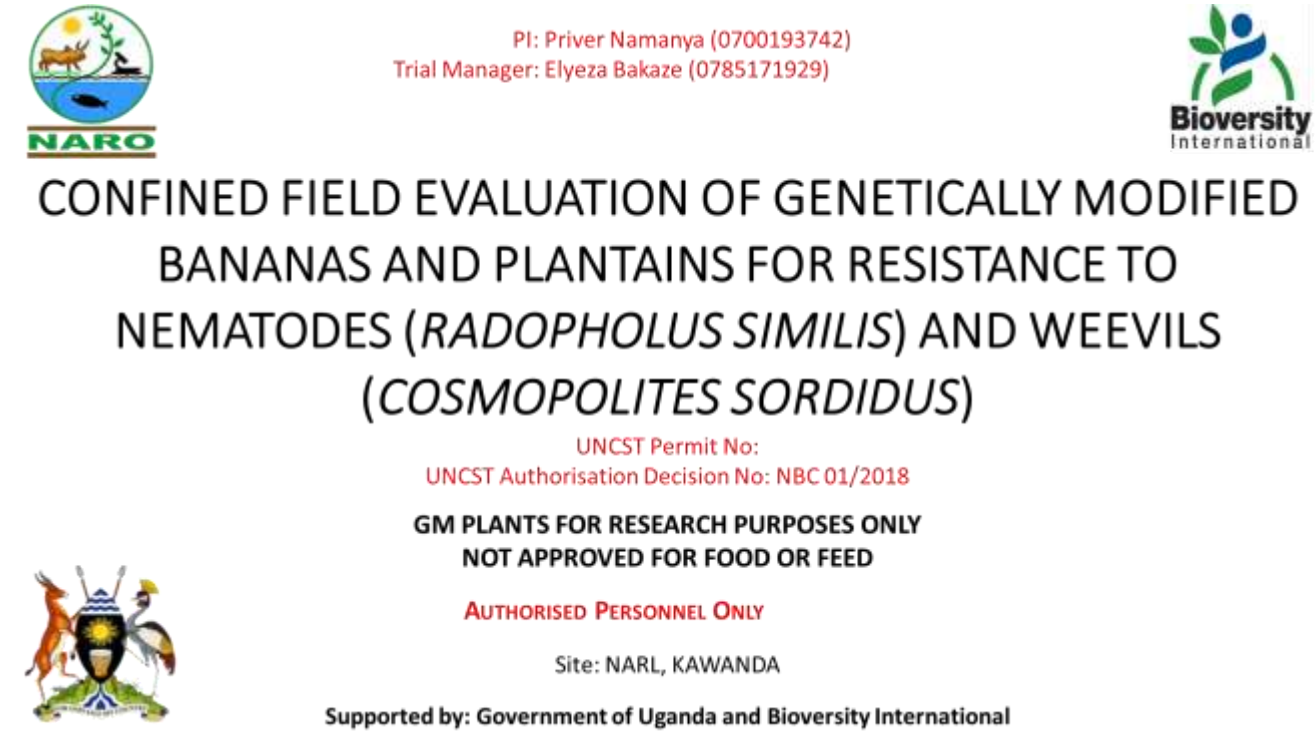

Figure 3.7: The regulatory signage to the CFT site.

Figure 3.8: The regulatory signage to the CFT site.

Therefore, in preparation for planting of selected transgenic and non-transgenic control lines, the project has conducted the following activities; (i) the CFT site and its facilities are now under 24/7 security surveillance with 3 security guards, (ii) the overgrown bush and shrubs in the field have been slashed and dry grass collected to provide mulch to the plants, (iii) garden tools and chemicals to be used in the site have been procured, (iv) construction of a water storage and supply network has commenced in the site, (v) construction of four incineration pits for disposal of in-field transgenic waste has commenced, (vi) constructed a pit-latrine, (vii) printing of a sign post to CFT site and in-field plant labels, (viii) manure, (ix) marking of the field and digging of planting holes.

3.5 Maintenance of inoculum for nematode and weevil populations

3.5.1 Establishment and maintenance of nematodes

Prior to planting, test plants will be inoculated with 2000 nematodes per plant to establish in the root system of the plants which will be subsequently transferred to the established field in the CFT. Therefore, pure *Radopholus similis* cultures were initiated and maintained to raise the required inoculum population of over 2,000,000 nematodes. This being done *in vitro* using carrot discs and *in vivo* in roots of potted plants of selected East African highland banana (EAHB) cultivars in the screen house (Fig. 2.9). To establish pure *R. similis* cultures, the project sampled banana roots for nematodes, identified, extracted and picked single *R. similis* nematodes from the extract. This was followed by introduction of *R. similis* pure inoculum on either carrot discs or clean potted banana plants.

Maintenance of pure nematode cultures has been achieved through regular sub culturing (4 - 6 weeks intervals) and periodically, *in vitro* cultures were transferred to potted banana plants to boost their infection of plant roots. The *in vivo*

cultures were also periodically transferred to new plants when nematodes damaged root system of the older plants. In order to achieve this milestone, laboratory consumables, chemicals and field supplies have been procured and delivered to the Nematology Lab.

Prior to planting of the CFT, the project will challenge the selected banana lines with *R. similis* to enable proper establishment of inoculum in the potted plants. Additionally, pre-planting soil sampling of the CFT will be conducted to identify and quantify parasitic and non-parasitic nematodes present in the experimental fields. Environmental biosafety studies on presence or absence of non-parasitic nematodes will provide an indicator on the effect of introduced transgenes to non-target organisms.

Figure 3.9: Maintenance of nematode inoculum *in vitro* on carrot discs (A) and *in vivo* in roots of banana plants (B).

3.5.2 Establishment and maintenance of nematodes

In order to facilitate post-planting inoculation, up to 40,000 weevils (*Cosmopolites sordidus*) are needed to have enough population for inoculation of the test plants in the CFT. At 4 to 6 months after planting when the plants have properly established in the field and attained reasonable size, they will be inoculated with weevils. The project has continued trapping weevils using banana corms in on-station banana fields and transferring them to the Entomology Lab for maintenance. In order to achieve this milestone, the following activities are performed routine weevil tapping, collecting corms, cleaning weevil culture, hatching out new weevil clones and their maintenance (Fig. 2.10).

Figure 3.10: In-field trapping, sexing and maintenance of banana weevils.

4. Germplasm collection and its management

The regional Banana Germplasm Collection Centre for the East and Central Africa (ECA), located in Mbarara district, Uganda (Figure 3.11), continue to house the different bananas have been assembled from Uganda, Kenya, Rwanda, Burundi, Tanzania Democratic republic of Congo, South Africa, India, Papaua New Guinea, Brazil, Honduras to capture a wider ecological and plant adaptation regimes. The collection at Mbarara is committed to the conservation of maximum variability of both local and exotic germplasm to enable the sourcing of important key traits to be used in the crop improvement programmes (Figure 3.12). This work is achieved through a) collecting and accessing unique and new germplasm b.) pre-breeding characterization, evaluation and identification of genotypes with maximum potential for resistance against pests, diseases and stresses in the environment as well as good agronomic and yield characteristics, c) dissemination of materials to different stakeholders and providing support to students and researchers through identification and describing of materials being used in the course of their studies.

This current Biotech project phase ensure that the germplasm management and conservation activities are conducted well. Figure 3.11 show Dr Nicolas Rous listening to Elias Oyesigye who was formally responsible for not only ensuring the documentation of the Mbarara collection but also maintenance, and then linking to the Musa germplasm Information system (MGIS). Elias is no longer undertaking his functions at the collection due budget constraints. There are however two research assistants and three casual laborers hired to carry out all activities for collection management and conservation.

Figure 3.11: Musa Germplasm collection at Mbarara

Figure 3.12: A selection of the germplasm found at Mbarara regional collection Centre

5. Human capacity

In preparation for the activities and to meet the milestones of the project activities, (i) "To evaluate nematode and weevil resistant banana products in confined field trials" and (ii) "To generate new technologies (genes) for Foc 1 resistance", the project has recruit new staff members. Three security guards have been recruited to offer 24/7 security surveillance of the CFT. The two fields, nematode and weevil will each have one field technician and two assistants each to manage the CFT activities. To generate new technologies (genes) for Foc 1 resistance, two research assistants with training and working experience in molecular biology, microbiology and bioinformatics have been recruited to build a team that will isolate and clone Foc 1 resistance genes.

Additionally, both technical and support project staff including laboratory, field and the security personnel underwent a stewardship training in 'Biosafety and Data Collection'. The project team recently underwent a training where select NBC members participated as resource persons. All new staff especially the security guards have been taken through the SOPs

and guidelines that govern management of CFTs. The team will continuously undertake refresher trainings for existing and new staff.

5.1. PhD studentships.

NARO coordinated students: This phase of the Project has two objectives, that is, (i) To evaluate and release nematode and weevil resistant banana products and a tracking system and (ii) To generate new technologies for Foc and emerging traits. This report has therefore highlighted the progress made thus far in objective (i). The Project has not been able to commence activities in Objective (ii) mainly due to the limited funds that were transferred to NARO which also affected the recruitment of PhD students. Additionally, the emergency of the COVID-19 pandemic has also affected commencement of preliminary activities.

Bioversity International coordinated students: The two students who initiated their research during phase three of the project are progressing well. A summary of their project activities is summarized below:

1. **Developing genetic tools for integrating drought tolerance in East African highland banana production systems (Moureen Nasamba)**

Breeding requires prior selection of appropriate male and female parents. Majority of farmers in the East African region, particularly in Uganda prefer EAHBs, which have been used as female parents in current breeding programs. However, most of these EAHBs are very sensitive to drought. Therefore, this study aims to contribute to the improvement of East African Highland bananas through identification and introgression of drought tolerance genes into the EAHB populations. Specifically, screening of germplasm is required to identify tolerant candidates, which can be used as male parents in subsequent crosses to develop F1 population(s), which will then be characterized by phenotyping and the breeding potential of selected candidates established. This study also aims to characterize the developed F1 population by genotyping in order to identify molecular markers linked to drought tolerance in banana. Putative drought tolerance genes identified in this study can be isolated, cloned and incorporated (individually or stacked) into farmer-preferred but drought sensitive genotypes using biotechnology techniques such as genetic engineering.

This study was initiated with literature review on Breeding banana for drought tolerance (**objective 1**). A manuscript has been published in refereed Journal, Plant Breeding, https://doi.org/10.1111/pbr.12812. Field survey to assess effects of drought on banana growth characteristics and coping strategies for farmers in Central Uganda was partially done (**objective 2**). Additional survey for banana farmers in Southwestern region of Uganda is to be complete by end of 2020. Sourcing and multiplication of genotypes for use in the screening experiment as part of **objective 3** on screening banana germplasm for drought tolerance to select candidate genotypes has been completed. Laboratory analyses to determine changes in biochemical compounds like proline, soluble proteins and free amino acids remains to be done. Developing F1 population(s) to be screened for drought tolerance is ongoing under **objective 4**. Phenotyping of F1 progeny under controlled screenhouse conditions and genotyping of F1 population(s) using Diversity Array Technology sequencing remains to be initiated in first quarter of 2021. Activities to achieve **objective 5**, to analyze the breeding potential of F1 progeny based on their pollen fertility will be initiated in the second quarter of 2021.

The Biotech project has limited funds to support the student for the research activities. Supplementary support funds are being sought.

2. **Characterization of the effects of banana root exudates on Fusarium oxysporum f.sp cubense race 1 in mixed cultivar systems (Georgina Mwaka)**

Previous studies suggest that banana root exudates break the dormancy of the hard-cased *Fusarium oxysporum* f.sp *cubense (Foc)* spores. However, there is still poor knowledge on *Foc* and the banana root system especially in EAHB. This study, therefore, seeks to determine the effect of mixed cropping on development of *Foc* incidence and severity in the susceptible banana cultivars in Uganda. This study aims to determine the composition of banana root exudates from

the resistant EAHB and their effect on *Foc* race 1 spore germination and hyphae development. The study will further determine the differential beneficial microbes between the resistant and susceptible varieties, influenced by mixed cropping and how these microbes affect *Foc*. Understanding the mechanisms through which mixed cropping and banana root exudates may minimize the impact of *Foc* in susceptible varieties, will not only provide strategies for disease management, but will also ensure continuity of these locally accepted *Foc*-susceptible varieties in Uganda. This study is hoped to form a baseline for genetic transformation of Foc-susceptible bananas with the target gene to enhance disease resistance and protection.

Baseline survey on banana-based cropping systems and Fusarium wilt incidence in Uganda has been partially completed for farms in south-western and north-western Uganda **(objective 1).** Farms in western and eastern Uganda, GIS mapping and statistical analysis are to be completed by end of 2020. For **objective** 2 on field and greenhouse evaluation of banana production systems on Fusarium wilt, selection of on-farm sites and planting materials was done and 6 farms were chosen from Luweero and Nakaseke. The on-farm and on-station trials at Kifu were set up and soil samples are being collected as well as disease progress monitored. The screen house trial is yet to be set up at Kawanda and Foc inoculum levels are yet to be determined using Qpcr as we still lack soil DNA extraction kits. Collection of root, rhizosphere and soil samples from the on-farm, on-station and screenhouse trials is yet to be done to achieve **objective 3** on Identification of rhizobiome and endophytome in mixed cropping systems. This would be followed by total DNA isolation of these samples. Isolation of biocontrol candidates such as *Trichoderma*, *Pseudomonas fluorescens* will also be conducted. These samples would need to be sent for sequencing and bioinformatics. Activities to achieve **objective 4** on analysis of root exudates from EAHB, Cavendish and susceptible varieties will be initiated in the second quarter of 2021.

The Biotech project has limited funds to support the student for the research activities. Supplementary support funds are being sought.

6. Construction of Tissue Culture laboratory at NARL, Kawanda

The project continues to focus on infrastructural development and maintenance in the quest to provide a solid basis for new scientific advances. The ever-increasing research activities, equipment and research personnel in tissue culture and biotechnology necessitate more lab space. To meet the demand, the Project is constructing a new Tissue Culture facilitate that will be completed by end of 2020 (Figure 3.13). The lab which is 750 square metres is aimed at contributing to the overall goal of NARL-NARO to bring about positive change in the livelihoods of producers by generating technologies for improving and sustaining productivity of the mandate commodities and systems. The laboratory will specifically contribute be generating over 5 million of tissue culture planting material for both banana and other horticultural crops.

Figure 3.13: Pictorial of the laboratory when complete

The laboratory is currently about 90% complete (Figure 4.14). The external works, installation of ACs and fencing remains to be done which are expected to be complete by end of December 2020.

(a)

(b)

(C)

(d)

e)

Figure 3.14: Photographs of the laboratory construction progress: (a) front view, (b) behind view, (c) verandah (d)/(e) Internal view

7. Project management

As has been the practice for this project in the last three phases, a Steering Committee (SC) to oversee the activities of this project in terms of technical guidance for the implementation was selected. The SC met in January and February 2020 to priorities project activities and agree on project budgets. The project has so far prepared two letters of agreements in favour of the project implementing partner, NARL- Kawanda for 2019 and 2020. The project has been unable to carry out a project review meeting for 2020 due to covid-19. The next project review and SC meeting will be conducted in June 2021.

8. Publications

Some of the results generated from the work done in the reporting period, have been developed into publications as indicated below:

1. Moureen Nansamba, Julia Sibiya, Robooni Tumuhimbise, Deborah Karamura, Jerome Kubiriba and Eldad Karamura, 2019. Breeding banana (*Musa* spp.) for drought tolerance: A review. Planting Breeding, 139 (4): 685-696, https://doi.org/10.1111/pbr.12812

9. Workplan

Project workplan	Pre-Implementation				2020				2021			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Objective 1: Evaluate and Release nematode and weevil resistant banana products and a tracking system.												
1.1 Select elite lines from CFT at Kawanda												
•Establish and maintain plants in glass and screen house facilities												
•Propose CFT site for NBC approval												
•CFT Application submitted and NBC approved												
•Establishment of CFT faciltities at NARL, Kawanda												
•Inspection and Approval of CFT facilities by NBC												
•Collect, Establish and maintain Nematode innoculum in potted plants												
•Collect, Establish and maintain Weevil innoculum in potted plants												
•Nematode innoculation (Pre-planting)												
•Planting of CFT at NARL, Kawanda												
•Weevil innoculation (Post-planting)												
•Primary molecular characterisation (PCR) - resistant lines												
•Weevil damage data												
•Nematodes population build-up /damage data												
•Agronomic and yield data collection												
1.2 Detailed molecular analyses to meet regulation requirements.												
•Southern blots to select lines with 1-3 copies and eliminate duplicate lines												
•PCR for presence of back-bone sequences.												
•Expression studies by qPCR and ELISA .												
•Gene insert analysis to ensure no new ORFs.												
1.3 Advance elite lines from MTL for commercial release.												
•In vitro multiplication of 5 selected lines												
•Establish and maintain 3 CFT sites: Kawanda, Mbarara, Bulindi-Hoima.												
•Weevil damage data												
•Nematodes population build-up /damage data												
•Agronomic and yield data collection												
•Determine fruit compositional data.												
•Collect environmental, allergenicity and toxicity data.												
•Collect data on Distinctness, Uniformity and Stability (DUS).												
•Consumer sensory acceptability data.												
•Compiling the Deregulation dossier												
•Develop a tracking and stewardship system to deliver products of integrity												
Objective 2: Generate new technologies for Foc and emerging traits.												
2.1 Gene isolation and cloning.												
•Maintain selected genotypes; Establish in tissue culture and wean in pots												
•Infection stages; prepare Foc inoculum, inoculate genotypes												
•Induce/Express target genes in banana cultivars.												
•Extract total RNA and genomic DNA.												
•cDNA synthesis												
•RNAseq / transcriptome analysis to identify genes up/down regulated.												
•Design PCR primers, identify target genes and their safety												
•Cloning, sequencing and analysis of target genes.												
•Build binary transformation vectors with Foc resistance genes.												
•Generate transgenic banana plants with Foc resistance genes.												
•PCR characterisation of putatively transgenic plants												
•Screen transgenic banana lines for resistance to Foc.												
2.2 Staff skill support - Gene annotation for RNA and DNA sequencing												
2.3 Tissue culture systems- Develop protoplas regenratation system -PhD												
2.4 Gene editing, annnotation for RNA and DNA sequencing -PhD												
3.1 Management of the Germplasm Collection												
3.2 Faciliting two PhD students from phase III to complete												
3.3 Pre-breeding Characterisation and Breeding												
3.4 Installations/Fittings for the Tissue Culture lab												
3.5 Steering committee meetings												
3.6 Planning/Review meetings												
3.7 Final project Evaluation												