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

NARO-Bioversity Banana Improvement Project

Annual Technical Report, 2017

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2. Excutive Summary

The project's overall objective is to improve East African highland bananas for resistance to pest and disease constraints (weevils, nematodes, bacterial wilt, black sigatoka and Fusarium wilt) by employing novel biotechnological approaches. This report highlights the research outputs of 2017, whose key activities included: a) Screenhouse evaluation of transgenic bananas for resistance to nematodes and weevils; b) Selection of consumer acceptable NABIO hybrids from on-farm trials; c) Establishing and maintaining NABIO embryonic cell suspension (ECS) lines; d) Developing molecular markers for nematode and weevil resistance; e) Developing Sukali Ndizi hybrids with resistance to Foc; f) Screening for somaclonal Kayinja lines with enhanced Foc resistance; and g) Germplasm collection, conservation and phenotyping for new traits (PVA and drought tolerance).

After proof-of-concept studies were completed, selected lines were re-initiated *in vitro* for bulking prior to planting in the CFT whose application has been duly submitted to the National Biosafety Committee for consideration. For Gonja Nakatansense, 24 and 22 transgenic lines were selected for resistance to nematode and weevils, respectively. Additionally, 67 and 41 transgenic lines of Nakitembe were selected for resistance to nematode and weevils, respectively. These lines are ready to go into the CFT trial at Kawanda in August.

We have previously reported on-farm trials of four NABIO hybrids (NABIO). After analysis of yield, agronomic evaluations in in diverse agroecologies, as well as the analysis of distinctness, uniformity and stability (DUS) data, two hybrids were submitted to the Ministry of Agriculture, Animal Industry and Forestry (MAAIF) Varietal Release Committee for commercial release to the farmers and consumers. Additionally, male buds of the four NABIOs were also initiated to establish regenerable embryogenic cell suspensions (ECS) to facilitate genetic engineering of multi-trait products. Ideal calli have been observed in the two hybrids, which is an indicator of progress to generating ECS from NABIOs. Additionally, the project developed two Sukali Ndizi hybrids resistant to Foc and with good consumer acceptability traits were selected and will be going into multi-location evaluations.

Screening for Foc resistance in Kayinja using somaclonal variation is ongoing with selected clones ready for field testing in disease hotspots. Validation of polymorphic primers to screen for weevil resistance among F2 segregating population is still on-going.

The banana germplasm at the Mbarara field germplasm collection has been maintained, with the addition of new genotypes to the collection. The continued characterisation of these accessions is being carried out to identify important traits (enhanced fruit PVA content and elevated tolerance against drought) and diploids that can be used conventional breeding activities. This will in turn produce multi-trait products that meet the needs of the farmers and consumer preferences. Additionally, through the project activities, a bulk of

infrastructure and human capacity has been built to consolidate the achievements of both phases of the project and lay a foundation for future activities.

3. Background

A healthy and ever increasing population growth rate demands increased food supply especially for the main staple food crops such as banana and cassava in many subsistence farming communities of Uganda. In the early 2000s Uganda adopted biotechnological approaches to complement already existing conventional methods to address bottlenecks to crop improvement and agricultural productivity. The goal was to ensure food security and incomes for the people of Uganda while reaping the full benefits of the revolution in biotechnology that was, and still is, transforming agricultural research and development around the world. Like other countries, Uganda stands to benefit from progress in agricultural industry. For example, since 1996 to date, the global area occupied by genetically engineered crops (GMO) has increased 100-fold from 1.7million to 175.2million hectares with estimated economic gains of US$58 billion for developing countries. In the case of GM soybean, maize, cotton and canola, productivity gains were 574 million tonnes with economic benefits worth US$167.8 billion. This reduced cost of production by US$15.4 billion saving 174 million hectares of valuable land under conventional crops *Brookes and Barfoot, 2015; https://www.pgeconomics.co.uk/pdf/2017globalimpactstudy.pdf.*

The choice of banana as a means of developing national biotechnological capacity was based on: i) the sterile nature of bananas which posed problems for conventional breeding and the minimum risk of 'gene flow' to weeds, local crops, or wild relatives of crops and hence negligible risk to the environment; ii) availability of banana wild relatives as sources of resistance genes in an approach called cisgenesis (as opposed to transgenesis where the resistance genes are obtained from other species other than the target crop); and iii) no other organisations (private or public) focused their research on East African Highland bananas (EAHB) and therefore, research would not compete with better-resourced organisations.

Therefore in 2002, the National Agricultural Research Organisation (NARO) of Uganda partnered with the International Network for the Improvement of Banana and Plantain (INIBAP) (currently Bioversity International) to achieve the following objectives:

Capacity building – to ensure that experts and critical infrastructure are made available to lead the research and development of innovations as they become available and to apply those of most value to the country.

Prioritisation – to ensure that resources are invested only in those technologies most useful to the country.

Effective transfer of technologies – to ensure that the country achieves ownership of the technology and that the tools and approaches are transferable to other important crops.

Networking – to ensure that the national team becomes aware of and can gain access to such innovations from national and international research collaborators in a timely manner.

3.1 Major deliverables

This report highlights continued progress of project activities, namely, development of transgenic banana with enhanced resistance to nematodes and weevil and release matooke hybrids ─the NARO-Bioversity hybrids (NABIOs). Other project activities focused on the development of molecular and biochemical markers for key traits (weevil and nematode resistance) and the development of bananas resistant to Fusarium Wilt (*Foc race 1*), targeting the local dessert industry. In addition, the project continued work on the conservation and characterisation of priority traits in the banana germplasm in the Mbarara *Musa* collection. The 2017 report highlights the following key achievements:

(a) Continued progress in somatic embryo mediated transformation system for EAHBs
(b) The development of NABIO embryonic cell suspension (ECS) lines,
(c) The evaluation of NABIO hybrids on-farm
(d) The multiplication of transgenic banana lines with enhanced protection against nematodes and weevils, in preparation for the Confined Field Trials on station
(e) The development of molecular markers for nematode and weevil resistance
(f) The development by cross-breeding of *Foc*-resistant apple-banana (Sukali Ndizi) hybrids
(g) The development of somaclonal Kayinja lines with Foc 1 resistance
(h) Genotypes with enhanced PVA levels and drought tolerance.

3.2 Project research focus for 2017

Key research areas included: i) Molecular characterisation of putatively transgenic Gonja and Nakitembe lines with *Cry6A*, *CpCYSΔ89* gene and their stack for resistance to weevils and nematodes under screenhouse conditions. ii) Developing ECS lines of three selected NABIO hybrids, iii) Developing molecular markers for weevil and nematode resistance, and iv) characterise banana germplasm at Mbarara for PVA levels and drought tolerance. Project objectives include:

(a) To develop transgenic Nakitembe and Gonja lines resistant to nematodes and weevils.

(b) To develop, evaluate and commercialise matooke, Sukali Ndizi and Kayinja hybrids with multiple resistance to weevils, nematodes, black Sigatoka, Foc and BXW.

(c) To develop transgenic NABIO hybrids with BXW resistance.

(d) To develop molecular and biochemical markers for weevil and nematode resistance.

(e) To conserve and characterise germplasm for priority traits useful in genetic improvement of EAHBs.

(f) To design, develop and strengthen SOPs, biosafety and stewardship tools for the project's transgenic products.

4. Summary of technical progress reports

4.1 Establishment of on-farm trial and release of NABIO hybrids

On-farm evaluation and selection are the final stage of any banana breeding process, leading to banana variety release. Upon endorsement by participating farmers and consumers, four NABIO hybrids: NABIO 0808 (M30), NABIO 0306 (M31), NABIO 0318 (M32) and NABIO 1011 (M33) met selection criteria (Tumuhimbise *et al*. 2016). In preparation for release to the farming community, key data regarding distinctness, uniformity and stability (DUS) has been collected by MAAIF and at least three test hybrids will be released by end of 2018. After going through evaluation, two NABIO varieties (Figure 1) were submitted to the Variety Release Committee for release to the farming community. We anticipate high uptake of these hybrids because of their high yield and consumer acceptability (Table 1).

NABIO808 (M30)	**NABIO306 (M31)**
	
-High yield of up to 54.5 t/ha/yr. -Resistant to black Sigatoka -Resistant to weevils -Resistant to nematodes -Acceptability = 5.5 out of 6	-High yield of up to 60.3t/ha/yr. -Resistant to black Sigatoka -Resistant to weevils, -Resistant to nematodes -Acceptability = 5.6 out of 6.

Figure 1: The matooke hybrids, NABIO 808 (M30) and NABIO 306 (M31), submitted for release. Panels below the pictures show the unique agronomic, yield and pest and resistance properties.

Table 1: Mean performance of the four hybrids against local check 'Mbwazirume' and hybrid check 'Kabana 6H' for fruit quality attributes.

	Fruit sensory traits (Hedonic scale: 1-6)				
Genotype	**Taste**	**Aroma**	**Mouth feel**	**Colour**	**Acceptability**
Kabana 6H	4.5	4.8	4.7	4.6	4.7
Mbwazirume	5.8	5.6	5.7	6.0	5.8
NABIO1011	5.0	5.1	5.1	4.8	5.1
NABIO306 (M31)	5.5	5.2	5.4	5.9	5.5
NABIO318	5.0	5.1	4.9	5.1	5.0
NABIO808 (M30)	5.6	5.4	5.6	5.9	5.6

Scale; 1-6, where: 1=Dislike very much, 2=Dislike, 3=Like moderately, 4=Like, 5=Like very much, 6=Like extremely. Study conducted across four sites (Mbarara, Hoima, Nakabango and Kawanda).

4.2 Establishing regenerable and transformable embryogenic cell suspensions

Many banana cultivars like Gonja, Nakitembe and the NABIOs lack some desirable traits. Improvement of such varieties for the traits they lack through genetic transformation requires highly regenerable target tissues (embryogenic cell suspensions, ECS). Two sources of explants (immature male flowers and scalps) are being used to generate ECS.

4.2.1 In vitro multiplication of 4 NABIO hybrids and establishment of a male bud multiplication field

Four NABIO hybrids (306, 308, 318 and 1011) were earmarked for establishment of ECS using immature male inflorescences. To establish a source of male flowers, the selected hybrids (four hundred plants per hybrid) were multiplied *in vitro*, weaned and planted in fields at NARL (200 plants) and on farm.

4.2.2 Initiate, establish and maintain ECS for EAHB hybrids- NABIOs from male flowers

The project plans to use genetic engineering to introgress traits of importance to develop multi-trait transgenic NABIOs. The first step for induction of embryogenic callus is the initiation of either immature flowers or highly meristematic tissues (scalps) on callus induction media (Figure 2). Over 100 scalp explants, hundreds of immature flowers are in culture with embryogenicity in some explants giving good

signs of ECS development. The embryogenic callus was initiated into liquid media with appropriate nutrient composition to generate embryogenic cells in a liquid suspension. Callus induction experiments were conducted on MS based media (MA1 or CHU N6 based media, B6), namely; i) MS based medium supplemented with 2,4 D alone, ii) MS based medium supplemented with auxin picloram alone, iii) MS or CHU N6 based medium supplemented with picloram and auxin 2,4D, iv) CHU N6 based medium, B6 supplemented with 2,4D and v) CHU N6 based medium supplemented with osmoticum poly ethyl glycol, PEG6000 and abcisic acid. Callus induction from immature flowers of NABIOs showed no cell proliferation. The cells look like the type II/III cells and cultures are being maintained for continous observation (Figure 3). Liquid culture NABIO 808_842 is still under observation, while the two single embryos initiated in liquid culture [842 and 859] have not produced the expected results.

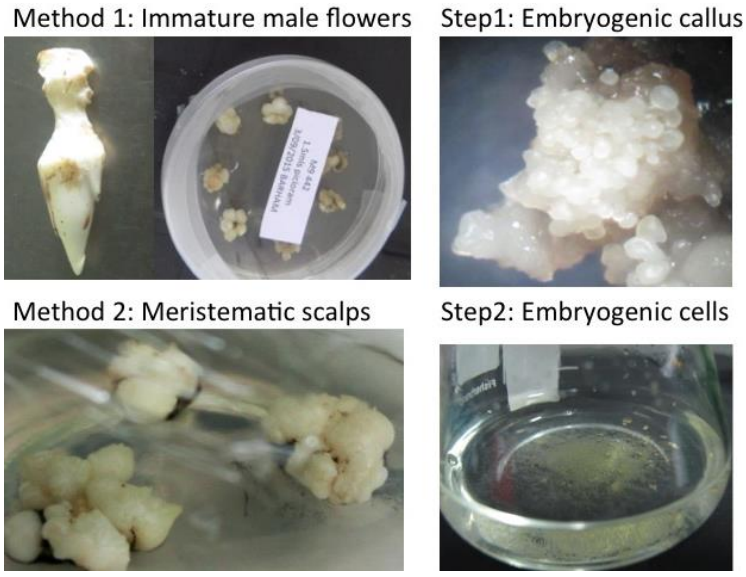

Figure 2: Illustration of two types of explant for callus induction from banana. Method 1 (immature male flowers) and Method 2 (scalps).

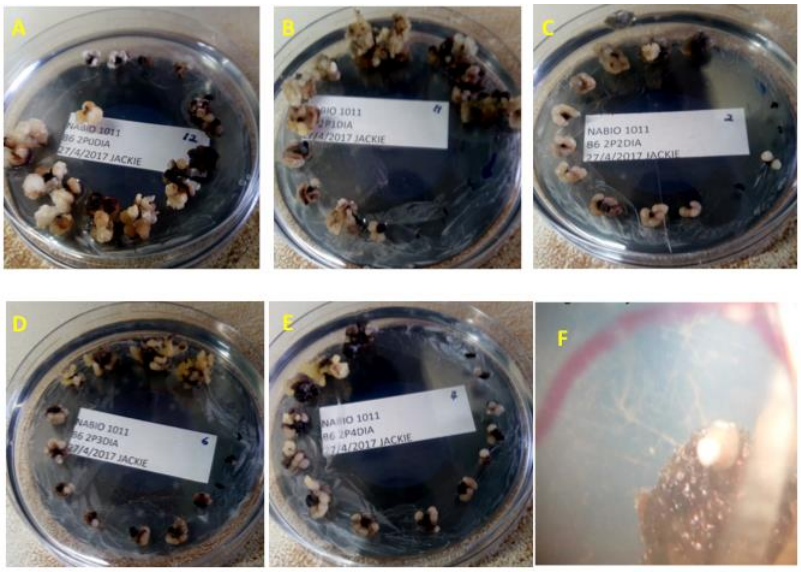

Figure 3: Representation of tissue reaction of immature male flowers of NABIO 1011.
Callus induction after 9 months on modified CHU N6 [B6) supplemented with 2mg/L picloram and **A**: 0mg/L 2,4-D; **B**: 1mg/L 2,4-D; **C**: 2mg/L 2,4-D; **D**: 3mg/L 2,4-D; **E**: 4mg/L 2,4-D; and **F**: Single embryo callus from NABIO 808 cultured on B6 supplemented with 1mg/L 2,4-D plus 0.6mg/L IAA.

4.2.2.1 Embryogenic cell suspension of cultivar Nakitembe

Immature male flowers of Nakitembe induced on B6 medium. Out of the seven Nakitembe cell lines, only three (NKT 732, 745 and 747) are still proliferating and regenerable and therefore still being maintained. Additionally, a new cell line NKT 846 was established and is ready for cryopreservation. Other ECS lines of Nakitembe (NKT 741, 742, 743, 745 and 747) and other cultivars; M9 (M9 498) and Gonja (GJ 665 and GJ 786) are under cryopreservation.

4.2.2.2 Retrieval of cell lines from cryopreservation

M9 (M9 498) established well in culture after 3 months, cells proliferated and multiplied but stopped multiplying after 9 months (Figure 4). Initial plating on MA3 for embryo formation was not successful but cultures are still under observation. Line NKT 747 was retrieved and established well in culture forming embryos when plated on MA3 with 65% regeneration rates while lines NKT 741, 742, 743 and 745 were retrieved but did not regenerate. Callus and cells for 'Gonja Nakatansense' were maintained for developing transgenic bananas with weevil and nematode resistance. Therefore, two cell lines of Gonja Nakatansense (GJ 665 and GJ 786) were cryopreserved but only GJ 786 is still proliferating and regenerable and therfore maintained in active culture.

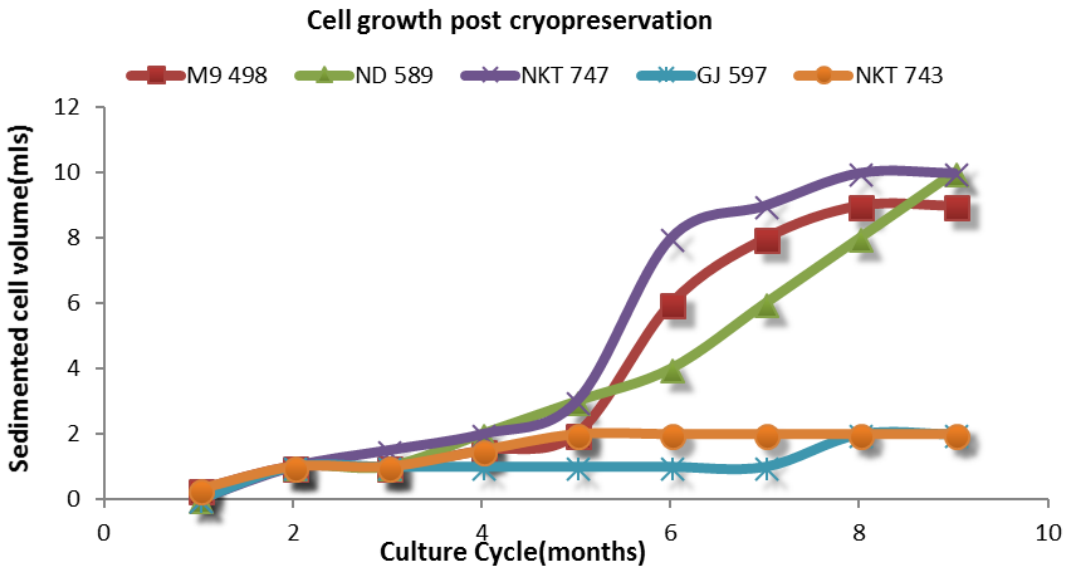

Figure 4: Cell growth of different cell lines post-cryopreservation showing hybrid M9, ND=Sukali Ndizi, NKT= Nakitembe and GJ=Gonja Nakatansense.

4.2.3 Callus induction using highly meristematic tissues (scalps)

Shoot tips from suckers are reduced in size, surface sterilised and cultured on 25ml MS based proliferation medium supplemented with 5mg/L BAP in baby jars for initial induction of multiple buds. The explants were cultured at 27 °C under a 14:10 hr light-dark lighting regime. After 3-4 months, multiple buds were transferred onto P4 Media consisting of MS based media with high concentration of cytokinin BAP at 100μM [22mg/L] or low concentration of TDZ at 10μM [2.02mg/L] to induce highly meristematic tissues with 'cauliflower-like' meristems called scalps (Figure 5). The cultures were then transferred to fresh P4 Media every 3-4weeks for 3-4 cycles until when "cauliflower-like" structures formed, whereby the leaf sheaths were highly reduced leaving naked meristems.

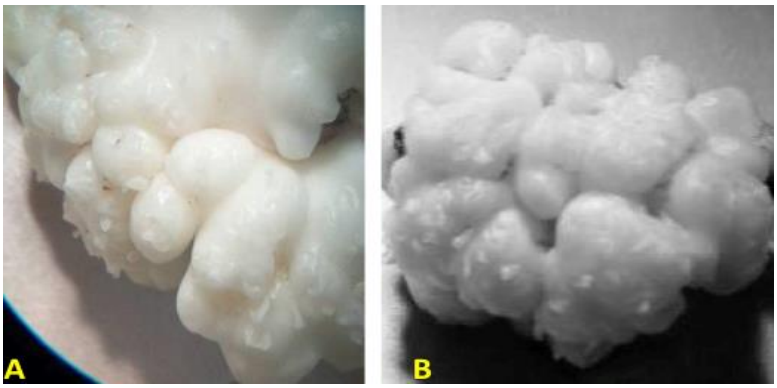

Figure 5: Highly meristematic tissues induced from NABIO 1011 cultured on MS based media supplemented with A: TDZ and B: BAP.

Over 600 scalps from three NABIO hybrids have been cultured onto ZZ callus induction media (Figure6). When cultured on ZZ media, the explants de-differentiated into yellow nodular callus and “friable-like tissues”. Some such tissues have been initiated in liquid media. However, no embryogenic tissues have been obtained yet from scalp cultures. Some of the tissues were initiated in liquid medium, five of NABIO Hybrids (NB808 842, NB306 845, NB1011 852, NB1011 854, NB808 855). However most of them have empty cells, none of them has generated regenerable embryogenic cells. Three of these (NB808 842, NB1011 852 and NB1011 854) are still under observation. A new set of fresh culture was started in January 2018 and these are undergoing the initial stages of bud induction prior to scalp induction (Table 2).

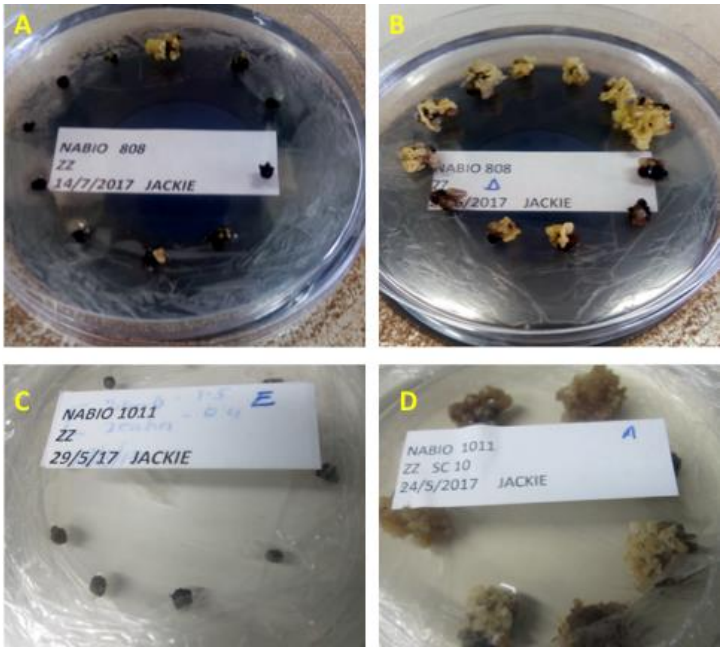

Figure 6: Illustrating scalps cultured onto ZZ callus induction media showing de-differentiation into yellow callus from A and B, NABIO 808 while C and D, NABIO 1011.

Table 2: Induction of fresh cultures of NABIOs

Genotype	**Level**	**No. of clusters/Buds**
NABIO M33 (1011)	Trf 2	81
NABIO M30 (808)	SC 1	96
NABIO M31 (306)	SC 1	192

The project selected scalps from the new fresh culture and these will be initiated directly into liquid media to generate embryogenic cell suspensions according to Sadik *et al*., 2007. A modification of ZZ medium at different concentration of zeatin (0.22mg/L ±] and or 2,4-D [1mg/L ±] will also be done.

4.3 Screenhouse evaluation of transgenic Nakitembe and Gonja Nakatansense for nematodes and weevils resistance

This work explores insecticidal and nematidical properties of *Bacillus thuringiensis* (Bt) crystalline (cry) proteins and plant proteinase inhibitors (cystatins). Transgenic lines with *Cry6A*, *Carica papaya* cystatin (*CpCYSΔ89*) and a stack of the two genes were developed. All transgenic lines, with the genes, were evaluated for weevil and nematode resistance under screen house conditions. During 2017, all transgenic Nakitembe and Gonja lines were screened in pots for resistance to nematodes and weevils. The selected transgenic lines were re-initiated back into tissue culture and bulked using *in vitro* multiplication and subsequently weaned and potted in the glasshouse. These plants will be planted in confined field trial (CFT) and screened for resistance to weevils and nematodes under field conditions.

4.3.1 Evaluation of transgenic Nakitembe and Gonja for nematode resistance

Using pot experiments, lines inoculated with nematodes were analysed for resistance to nematodes. Protection of the roots against nematodes was calculated from the necrosis index (NI) using the formula: 100 - (NI of transgenic line/NI of non-transformed Nakitembe*100). Transgenic plants exhibited protection range between 0 and 100% but non-transgenic controls had 0-13% while resistant check KM5 ranged between 83 - 100%. Based on 100% protection, selected transgenic lines are summarised in Tables 3-6.

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

Transgene	Line	Copies targeted	Copies potted
CpCYSΔ89	16	30	11
CpCYSΔ89	33	30	9
CpCYSΔ89	35	30	19
CpCYSΔ89	37	30	22
CpCYSΔ89	50	30	2
CpCYSΔ89	139	30	13
Cry6A	37	30	0
Cry6A	45	30	0
Cry6A	61	30	0
Cry6A	96	30	19
Cry6A	97	30	42

CpCYSΔ89 + Cry6A	5	30	28
CpCYSΔ89 + Cry6A	17	30	9
CpCYSΔ89 + Cry6A	20	30	19
CpCYSΔ89 + Cry6A	23	30	34
CpCYSΔ89 + Cry6A	26	30	27
CpCYSΔ89 + Cry6A	27	30	12
CpCYSΔ89 + Cry6A	28	30	0
CpCYSΔ89 + Cry6A	48	30	11
CpCYSΔ89 + Cry6A	57	30	33
CpCYSΔ89 + Cry6A	96	30	20
CpCYSΔ89 + Cry6A	115	30	27
CpCYSΔ89 + Cry6A	116	30	31
CpCYSΔ89 + Cry6A	134	30	18
24 lines			

Table 4: Selected transgenic lines of Nakitembe (AAA) with *CpCYSΔ89* for resistance to nematodes *Radopholus similis*

Transgene	Line	Copies targeted	Copies potted
CpCYSΔ89	3	30	0
CpCYSΔ89	5	30	0
CpCYSΔ89	8	30	22
CpCYSΔ89	27	30	20
CpCYSΔ89	28	30	25
CpCYSΔ89	34	30	6
CpCYSΔ89	35	30	3
CpCYSΔ89	52	30	11
CpCYSΔ89	55	30	20
CpCYSΔ89	60	30	7
CpCYSΔ89	86	30	0
CpCYSΔ89	91	30	80
CpCYSΔ89	97	30	11
CpCYSΔ89	109	30	5
CpCYSΔ89	115	30	14
CpCYSΔ89	120	30	0
CpCYSΔ89	123	30	40
CpCYSΔ89	154	30	4

CpCYSΔ89	164	30	21
CpCYSΔ89	171	30	18
CpCYSΔ89	177	30	15
CpCYSΔ89	178	30	1
CpCYSΔ89	179	30	31
23 lines			

Table 5: Selected transgenic lines of Nakitembe (AAA) with *Cry6A* for resistance to nematodes *Radopholus similis*

Transgene	**Line**	**Copies targeted**	**Copies potted**
Cry6A	10	30	24
Cry6A	15	30	6
Cry6A	18	30	17
Cry6A	38	30	24
Cry6A	39	30	34
Cry6A	40	30	0
Cry6A	49	30	12
Cry6A	62	30	2
Cry6A	90	30	0
Cry6A	97	30	35
Cry6A	103	30	4
Cry6A	106	30	10
Cry6A	116	30	13
Cry6A	140	30	26
Cry6A	161	30	0
Cry6A	163	30	3
Cry6A	176	30	23
Cry6A	189	30	29
Cry6A	191	30	12
Cry6A	202	30	15
Cry6A	242	30	23
Cry6A	248	30	28
22 lines			

Table 6: 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	30	7
CpCYSΔ89 + *Cry6A*	30	30	8
CpCYSΔ89 + *Cry6A*	31	30	1
CpCYSΔ89 + *Cry6A*	35	30	13
CpCYSΔ89 + *Cry6A*	36	30	3
CpCYSΔ89 + *Cry6A*	38	30	11
CpCYSΔ89 + *Cry6A*	44	30	10
CpCYSΔ89 + *Cry6A*	48	30	1
CpCYSΔ89 + *Cry6A*	50	30	2
CpCYSΔ89 + *Cry6A*	51	30	49
CpCYSΔ89 + *Cry6A*	54	30	10
CpCYSΔ89 + *Cry6A*	57	30	5
CpCYSΔ89 + *Cry6A*	71	30	6
CpCYSΔ89 + *Cry6A*	105	30	9
CpCYSΔ89 + *Cry6A*	109	30	5
CpCYSΔ89 + *Cry6A*	120	30	10
CpCYSΔ89 + *Cry6A*	127	30	33
CpCYSΔ89 + *Cry6A*	131	30	12
CpCYSΔ89 + *Cry6A*	137	30	1
CpCYSΔ89 + *Cry6A*	142	30	3
CpCYSΔ89 + *Cry6A*	152	30	3
CpCYSΔ89 + *Cry6A*	153	30	9
22 lines			

4.3.2 Evaluation of transgenic Nakitembe and Gonja for weevil resistance

Using pot experiments, lines inoculated with weevils were analysed for resistance. Protection of the roots against nematodes was determined by observing corm damage which was scored using “Percent Coefficient of Infestation” (PCI). Based on 100% protection, transgenic lines with 90-100% protection were selected (Tables 7-9). Additionally, wild-type controls (Table 10) will be planted in the CFT in September 2018, for comparison with the transgenic lines.

Table 7: Selected transgenic lines of Gonja (AAB) for resistance to banana weevil *Cosmopolites sordidus*

Transgene	Line	Copies targeted	Copies potted
CpCYSΔ89	12	30	12
CpCYSΔ89	39	30	37
CpCYSΔ89	44	30	11
CpCYSΔ89	48	30	17
CpCYSΔ89	50	30	7
CpCYSΔ89	56	30	18
CpCYSΔ89	72	30	9
CpCYSΔ89	118	30	0
CpCYSΔ89	137	30	28
CpCYSΔ89	138	30	18
CpCYSΔ89	144	30	0
CpCYSΔ89	145	30	1
CpCYSΔ89	147	30	10
CpCYSΔ89	179	30	0
CpCYSΔ89	189	30	18
Cry6A	74	30	8
CpCYSΔ89 + *Cry6A*	14	30	17
CpCYSΔ89 + *Cry6A*	24	30	15
CpCYSΔ89 + *Cry6A*	72	30	5
CpCYSΔ89 + *Cry6A*	101	30	22
CpCYSΔ89 + *Cry6A*	118	30	12
CpCYSΔ89 + *Cry6A*	264	30	7
22 lines			

Table 8: Selected transgenic lines of Nakitembe (AAA) with *Cry6A* for resistance to banana weevil *Cosmopolites sordidus*

Transgene	Line	Copies targeted	Copies potted
Cry6A	43	30	19
Cry6A	46	30	20
Cry6A	51	30	21
Cry6A	53	30	18
Cry6A	63	30	33
Cry6A	70	30	26
Cry6A	90	30	18
Cry6A	96	30	21
Cry6A	112	30	25
Cry6A	117	30	22
Cry6A	142	30	0
Cry6A	143	30	59
Cry6A	145	30	35
Cry6A	149	30	28
Cry6A	151	30	13
Cry6A	158	30	29
Cry6A	182	30	32
Cry6A	186	30	6
Cry6A	187	30	21
Cry6A	189	30	6
Cry6A	194	30	39
Cry6A	197	30	33
Cry6A	212	30	23
Cry6A	231	30	30
Cry6A	242	30	37
Cry6A	256	30	29
Cry6A	270	30	28
	27 lines		

Table 9: 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	58	30	15
CpCYSΔ89	90	30	24
CpCYSΔ89	168	30	23
CpCYSΔ89	288	30	18
CpCYSΔ89 + *Cry6A*	28	30	20
CpCYSΔ89 + *Cry6A*	52	30	42
CpCYSΔ89 + *Cry6A*	107	30	41
CpCYSΔ89 + *Cry6A*	152	30	21
CpCYSΔ89 + *Cry6A*	157	30	18
CpCYSΔ89 + *Cry6A*	159	30	13
CpCYSΔ89 + *Cry6A*	181	30	22
CpCYSΔ89 + *Cry6A*	212	30	39
CpCYSΔ89 + *Cry6A*	216	30	20
CpCYSΔ89 + *Cry6A*	220	30	15
14 lines			

Table 10: Controls of Nakitembe (AAA), Gonja (AAB) and Yangambi KM5

Cultivar	Phenotype	Copies targeted	Copies potted
Nakitembe	Non-transgenic	30	
Gonja	Non-transgenic	30	
KM5	Non-transgenic	30	
Nakitembe	GUS-transgenic	30	

In preparation for assessment of transgenic plants under CFT conditions, CFT application filed to NBC for approval during the June 2018 meeting. Additionally, the proposed site (Figure 7) was approved by the NBC and will soon be fenced off with chain-link fencing for approval as a CFT site. A layout of the CFT to cater for the two fields (weevil and nematodes) (Figure 8) has been completed and planting is scheduled for June 2018. Upon planting, plant growth, nematode and weevil damage, yield and molecular characterization data will be collected from plants in the CFT.

Figure 7: The proposed CFT site after clearing and ploughing.

Figure 8: Location and layout of the proposed site for the CFT (inclusive of nearby physical features).

4.4 Breeding for Fusarium wilt resistance

4.4.1 Screening for Foc resistance in somaclonal Kayinga lines

Fusarium wilt (*Fusarium oxysporum cubense,* race 1; *Foc*) is a major fungal disease, which causes total yield loss in a juice-banana variety 'Kayinja'. Since fungal spores persist for long in infected soils, it is important to develop resistant lines. As part of developing long-term and practical control strategy of the disease, somaclonal variation strategy is being assessed to screen for genotypes with resistance in Kayinja. Starting sucker material was collected on station at NARL-Kawanda and a farmer's field in Sayi-Matugga. From multiple *in vitro* sub-culture cycles, the project has weaned plants from cycles 7, 9, 11, 12, 14 and 15, and examined them for the presence or absence of corm discolouration as the indicator for *Foc* resistance/susceptibility. Pot experiment screening of 3618 plants showed *Foc* resistance in 34 plants with 0 - 10 % corm discolouration. The 34 plants (lines) in pots were further screened from which 9 plants (lines) (Kay 09, Kay 23, Kay 29, Kay 30, Kay 40, Kay 154, Kay 366, Kay 383 and Kay 457) showed 0% corm discolouration and were bulked *in vitro*. Twenty copies of each line will be further examined for resistance under field conditions in a *Foc* hot spot at NARL-Kawanda and planting planned for August 2018.

4.4.2 Developing Foc*-resistant apple banana (Sukali Ndizi) hybrids*

Dessert bananas form the bulk of the global banana trade with a value of more than US$ 4 billion annually (FAOSTAT, 2013). However, they are less than 10% of the banana cultivar profile in Uganda largely due to Fusarium wilt, which is very difficult to control without resistant varieties. Although introduced FHIA varieties have good resistance to *Foc*, they have poor consumer acceptability. The main purpose of this study was to conventionally breed for *Foc* resistance apple banana (Sukali Ndizi) hybrid, which maintains cultivar fruit qualities. This was achieved through maintenance and expansion of progenies, ploidy and genomic analysis of progenies, field evaluation of progenies for *Foc* resistance, collecting agronomic data of the progenies, physico-chemical analysis and sensory evaluation of the promising resistant progenies. The data collected included: i) *Foc* response during the growing session and at harvest; ii) agronomic data of the progenies; iii) yield data at harvest using yield related parameters; iv) sensory evaluation using a panel of judges when the fruits a fully ripe; and v). physico-chemical analysis with two promising candidate hybrids.

Notably the selected two new apple banana hybrids (NAMU1 and NAMU2) and a *Foc* race 1 susceptible but consumer-preferred local commercial Sukali Ndizi (the parent to the two hybrids) are currently being evaluated at NARL-Kawanda. Preliminary results show that the bunch yields of the two new apple banana hybrids were over 50% higher than those of check cultivar (KM5) (Fig 9). NAMU1 and KM5 showed no

corm and pseudostem symptoms for *Foc*. However, NAMU2 showed mild symptoms on corms when compared to the parent Sukali Ndizi with severe symptoms on both corm and pseudo stem. The ripe fruit pulp texture, taste, smell and acceptability of NAMU1 and NAMU2 were not significantly different from those of the parent. Therefore, it is proposed that the two hybrids will be further evaluated on-farm and in the market for commercial release. This will be a major milestone towards the development of dessert banana industry in Uganda.

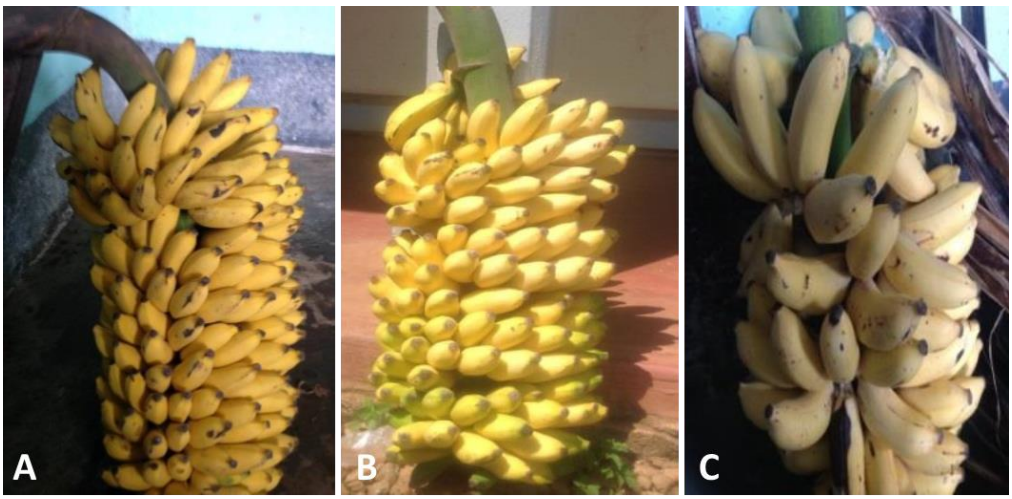

Figure 9: New apple (Sukali Ndizi) hybrids to *Foc* race 1, where A, NAMU1; B, NAMU2 are hybrids and C, parent Sukali Ndizi.

4.4.3 Developing molecular markers for nematode and weevil resistance

Improvement of banana through conventional breeding is constrained by long crop duration (up to 2 two years) and requirement of large space which increases the expenses for evaluation of progenies. Such expenses and time can be saved by using Marker Assisted Selection for desired traits in genotypes. From previous studies (Kabiita, 2012), a field of F2 segregating population (242 genotypes) for weevil resistance and their two diploid parents *Musa acuminata* Subsp banksii (Kasaska) and *Musa acuminata* Subsp microcarpa (Borneo) in addition to the F1 accessions was established at NARL-Kawanda. The main objective of this activity was to optimise and validate the available nematode resistance SSR markers using F2 segregating populations of crosses between weevil susceptible 'Kasaska' and resistant 'Borneo'. As part of establishing a PCR based protocol, a better resolution gel (metaphor) was optimised and used to enhance the clarity, detection and separation of small DNA fragments, to come up with SSR markers. Using PCR, 33 SSR primers were used to screen for nematode resistance in parental DNA and F1 population. Only primers showing amplification in parental and F1 DNA were selected for further

screening with 13 amplifying parental DNA with differentiation between the two parents (susceptible and resistant) where 4 produced a single band while 9 had 2 or 3 bands.

Notably, using selected primer sets showed an extra band in some F2 population genotypes but was absent in both parents. Further study such as sequencing analysis of the unique bands could reveal more information about the genotype identity. Some genotypes show dominance across all the markers. Previous phenotypic information could not sufficiently to distinguish between resistance and susceptibility. Therefore, these genotypes will be multiplied *in vitro* and screened for both weevil and nematode resistance in pots under screenhouse conditions. *In vitro* initiation propagation of the promising F2 genotypes and parents has already started. Results will then be related with the genotypic information obtained with the markers above. The segregated population will be evaluated for nematode resistance under screenhouse conditions using pot experiments. The data will then be validated with the markers in order to confirm the markers for nematode resistance.

4.5 Germplasm conservation for future improvement of EAH bananas

The primary aim of *Musa* germplasm conservation and characterization is to provide information for appropriate use in supporting breeding work and farmers' livelihoods. There is need for effective selection of prospective male and female parents in the breeding process, depending on the priority traits which have been identified by breeding programs. The priority traits being worked on in this programme include among others: high nutrition value mainly PVA content, tolerance to drought, dwarfness and resistance to pests and diseases (and later delayed ripening). The objectives of this study were to: i) access and conserve maximum diversity of *Musa* species and utilize them to isolate those with desired traits by breeders and ii) characterise and conduct preliminary phenotyping of the genotypes: for Provitamin A content, tolerance to drought and dwarfism. The project's main activities in 2017 were to:

i) Access desired genotypes from ITC and other breeding programmes
ii) Identify desired accessions (with high PVA content, drought tolerant and dwarfism) from the collected materials
iii) Conduct preliminary phenotyping of the genotypes for Provitamin A content
iv) Establish a crossing block for the selected male genotypes of high Provitamin A content and selected best female parents
v) Detect dwarfism among the East African Highland bananas and vi) compiling characterisation data for the data base.

4.5.1 Access to germplasm

Thirty-nine genotypes (Table 11) were accessed and will be added to the existing germplasm collection to enable further selection of materials with desired traits by the breeders. Other highlights included planting of two high PVA content genotypes (*Pisang Jari Buaya* and TMB2 X 9722-1) in a crossing block to act as male parents to enable the generation of high PVA content population (see Breeding Report). A method for evaluating dwarf cultivars among 238 EAHBs (*Musa* AAA-EA) accessions was also developed. Of the 13 traits used to study the 238 accessions, only two traits, leaf length-width ratio and plant height-girth ratio consistently had minimum variability across the accessions in relation to Nakyetengu the known dwarf East African Highland banana (EAHB) and hence the two traits can be used in studying dwarfism in bananas. Apart from Nakyetengu, breeders can select cultivars that have leaf length-width ratio not exceeding 2.5 and the plant height -girth ratio not exceeding 3.6 as female parents. A scatter plot of 238 (EAHB) accessions with 31 Cavendish based on the lamina length - width ratio and plant height - girth ratio is presented. With plant height - girth ratio below 3.6 and leaf length-width ratio below 2.5, most accessions (genotypes in oval shape) (Figure10) clustered with Nakyetengu which is one of the female parent in the pre-breeding block.

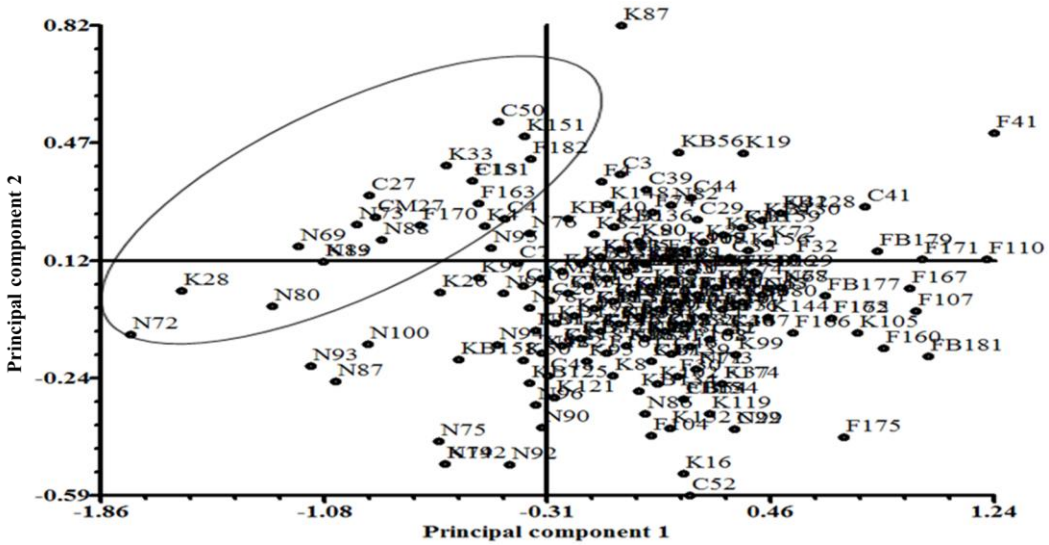

Figure 10: A scatter plot of 238 accessions with 31 Cavendish based on the lamina length-width ratio and plant height-girth ratio.

Serial Number	Accession Name	Genome group	Origin of material	Date received	Status of material
1	BRS Victoria	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
2	BRS Preciosa	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
3	BRS Prata-Ana	AAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
4	BRS Japira	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
5	BRS Garantida	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
6	BRS Tropical	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
7	BRS Pacovan	AAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
8	BRS Platina	AAAB	Embrapa breeding programme	30/06/2015	Block one of the ECAR collection
9	BRS Princesa	AAAB	Embrapa breeding programme	30/05/2015	Block one of the ECAR collection
10	Sukali Ndizi	AAB	NARO-breeding programme	30/06/2015	Block one of the ECAR collection
11	Tudlo Tumbaga	AA	Micronutrient deficiencies Project	05/11/2017	Block one of the ECAR collection
12	Truncata	AA	ITC 0393, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
13	Hung Tu	AA	ITC 0601, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
14	Kekiau	AA	ITC 0776, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
15	Gorop	AA	ITC 0778, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
16	Pama	AA	ITC 0797, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
17	Vunamami	AS	ITC 0801, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
18	Ato	AA	ITC 0820, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
19	Sepi	AA	ITC 0849, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
20	Skai	AS	ITC 0883, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
21	Sira	AA	ITC 0907, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
22	Gilasalasa	AA	ITC 0932, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
23	Yangun Yefan	AA	ITC 0984, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
24	Tango	AA	ITC 1012, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
25	Terema	AA	ITC 1214, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
26	Yalumia	AA	ITC 1220, Belgium	13/09 /2017	*In vitro*-NARO Breeding Programme
27	Nakawere	AAA-EA	On Farm (Kiboga)	15/11/2017	Block three of the ECAR collection
28	201071 K	AB	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
29	Line 11-17 K	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
30	Line 10-9 K	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
31	ND 2050	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
32	ND 2161	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
33	ND 2121	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
34	ND 2194	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
35	Slamury	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
36	ND 2105	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
37	ND 2099	AB ?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
38	Wambo	AA	NARO-breeding programme	08/12/2017	Block one of the ECAR collection
39	ND 2131	AB?	NARO-breeding programme	08/12/2017	Block one of the ECAR collection

Table 11: Materials accessed from other breeding programmes.

4.5.2 Phenotyping for PVA and drought tolerance

Screening banana germplasm for PVA and drought tolerance were initiated in October 2016 with one hundred and eighteen genotypes including 109 diploids, 6 NABIO hybrids and M9 to be analysed for PVA content (see 2016 Annual Biotech Report) (Table 12). Significant variability in PVA has been observed in 12 out of 106 banana accessions to be phenotyped. The highest PVA has so far been recorded in

diploid TMB2X 9722-1, followed by *Pisang Jari Buaya* while the lowest was observed in *Khai Thong Ruang* and Butuhan Intermediate Apex. It is important to note that a good phenotyping plan is a prerequisite for any plant improvement programme for targeted traits.

Table 12: List of banana genotypes and cultivars selected for fruit PVA (in beta-carotene equivalent, µg/g DW) content analysis

Sample ID	Sample name	BCE-FG (µg/g DW)	BCE-FR (µg/g DW)
MBA-001	*Musa balbisiana*	6.7	9.6
MBA-002	NABIO 318	9.3	11.4
MBA-003	NABIO306	8.1	12.3
MBA-004	TUUGIA 610	2.8	7.0
MBA-005	*Pisang Jari Buaya*	27.0	31.0
MBA-006	NABIO 1011	8.8	12.9
MBA-007	Kamynyila	2.6	3.4
MBA-008	NABIO1009	6.9	6.5
MBA-009	TMB2X 9722-1	32.2	39.5
MBA-010	Njuru	4.0	5.4
MBA-011	*Butuhan Intermediate Apex*	1.4	2.6
MBA-012	*Khai Thong Ruang*	1.4	1.8
MBA-013	NABIO 1117	6.5	7.4

5. Draft Publications

Efficacy of Bt (Cry6A) and Papaya Cystatin transgenes in suppressing weevil damage in plantain cv “Gonja-Nakatansese” AAB Genome. David Talengera, Charles Mwami, Tony Tazuba, Doreen Amumpaire, Priver Namanya, Jerome Kubiriba, W.K. Tushemereirwe, Geofrey Arinaitwe and Eldad Karamura (Draft form).

6. References

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Graham Brookes & Peter Barfoot (2017). GM crops: global socio-economic and environmental impacts 1996- 2015 published by PG Economics Ltd 2017 [https://www.pgeconomics.co.uk/pdf/2017globalimpactstudy.pdf]

SADIK K., ARINAITWE G., SSEBULIBA JM., GIBSON P., LUGOLOBI C. and MUKASA SB. (2012) African proliferation and shoot recovery among the east african highland banana. *African Crop Science Journal*, Vol. 20, Issue Supplement s1, pp. 67 - 76 ISSN 1021-9730/2012