DRAFT ANNUAL REPORT 2013/14: NATIONAL BANANA RESEARCH PROGRAMME-NARL

INTRODUCTION

The agriculture sector Development Strategy and Investment Plan (DSIP) of the government of Uganda seeks to increase food security through increasing agricultural production and productivity. Food security is a key priority for t population in Uganda at 34.9 million (National Census-UBOS, 2014) and set to keep increasing at annual rate of 3%. The need to feed this population puts increasing pressure on the fixed land and is aggravated by the increasingly degraded environment, pests and diseases. Such declining and variable environments require crops adapted to a wide range of agro-ecologies such as banana.

Banana has been consistently ranked as number one crop by the farming communities in Uganda as it is an indispensable part of life for more than a half of the population. Its perennial nature coupled with an all-year-round fruiting character makes it an ideal crop for household incomes, food and nutrition security. As a climate change ameliorating agent, it picks more CO_2 from the atmosphere than most other food security crops and contributes amounts of soil organic carbon comparable to that of woody species. Its dense leaf canopy and extensive root system contribute to a stable agro-ecological system. In fact, the Uganda's matooke market unutilized potential within in urban areas is estimated at 50% and that of dessert banana at over 90% in East and Central African region. Despite these benefits, banana has suffered from pests (banana weevils and nematodes), diseases (*Fusarium* wilt, bacterial wilt and black Sigatoka) and more recently, drought stress. The core responsibility of the National Agricultural Research Laboratories' Banana Research is to generate and promote technologies that increase and banana productivity and utilization for the Ugandan banana dependent communities.

Objectives of the National Banana Research Programme

The main goal of the National Banana Research Programme above is accomplished through the following specific objectives:

1. Improve bananas for pest/disease resistance and fruit quality through conventional and molecular breeding;
2. Generation and promotion of integrated technologies for banana bacterial wilt and other stresses.

Research thrust

The Research for Development activities are driven by developing resistance to pests, diseases and improved fruit quality using both conventional and molecular breeding. It has been recognized by the banana stakeholders that the products of the banana improvement activities may take long to deliver. In the short run, we focus on generation and promotion of cultural practice based technologies for integrated management of banana pests and diseases, with particular emphasis on banana bacterial wilt (BBW) control.

KEY ACHIEVEMENTS

CONVENTIONAL BANANA BREEDING

Breeding (conventional and molecular) has one of the highest rates of return among the investments in agricultural research. National Banana Research Programme has made significant breeding achievements, ranging from generation of new hybrids to selection and release of new matooke hybrids meeting consumer preference. Below are the achievements made during this reporting period.

- Over 10,000 banana seeds were generated. About1000 new banana hybrids were established in Early Evaluation Trial (EET) at NARL-Kawanda.
- Fifteen new banana hybrids were selected from existing EETs for advancing to Preliminary Yield Trials (PYT) in the next planting season of 2015. They included: 9019K, 252K, 274K, 6360K, 247K, 203K, 236K, 13250S-1, 12658S-1, 273K, 2052011K, 20K, 20101K, 2010102K and 35K.
- The program evaluated 19 new banana hybrids in PYTs at NARL-Kawanda, Bulindi-Hoima, Nakabango-Jinja and MBAZARD-Mbarara. Of the 19 hybrids being evaluated, three [NB0808 (M30); NB1009 (M31) and NB0614 (M32)] have so far been identified as being best in terms of yield, black Sigatoka resistance and overall acceptability. Their yield is over 40 t/ha/yr and, youngest leaf spotted at flowering is over 7.0 with an overall consumer acceptability of over 4.0 points.

Plate 1. Promising matooke hybrid (M30)

Table 1.Matooke hybrids selected for advanced onfarm multilocational trials

Hybrid	T/Ha/Yr	Accept.
30	**53**	**5.0**
M31	43	-
M32	41	-
M29	32	3.5
M28	40	4.3
M27	39	4.8
M25	38	4.5
Kibuzi	**26**	**4.8**

- Six banana hybrids (M19, M20, M25, M27, M28 and M29) were selected from on farm trials in Mpigi and Kimenyedde for further evaluation in advanced multilocational trials. Hybrids are already micro-propagated for planting in the first rainy season of 2015.
- Banana hybrids Kabana 3H (FHIA 17), Kabana 6H (M9) and Kabana 7H (M2) that were officially released to the farming communities are being promoted to all regions of Uganda. Over 600 demonstration plots were established in northern, western, central and eastern Uganda (Figure 1). The plots are already being utilized as banana learning resource centers and also act as mother-gardens for easy access of planting materials of the released hybrids by the surrounding communities. Individual farmers in Northern Uganda, especially in Pader district have already benefited from these established demonstration plots by selling suckers and banana bunches. Future promotional activities for banana crop management technologies, additional conventionally bred improved hybrids and genetically modified banana varieties will be centered on these plots.

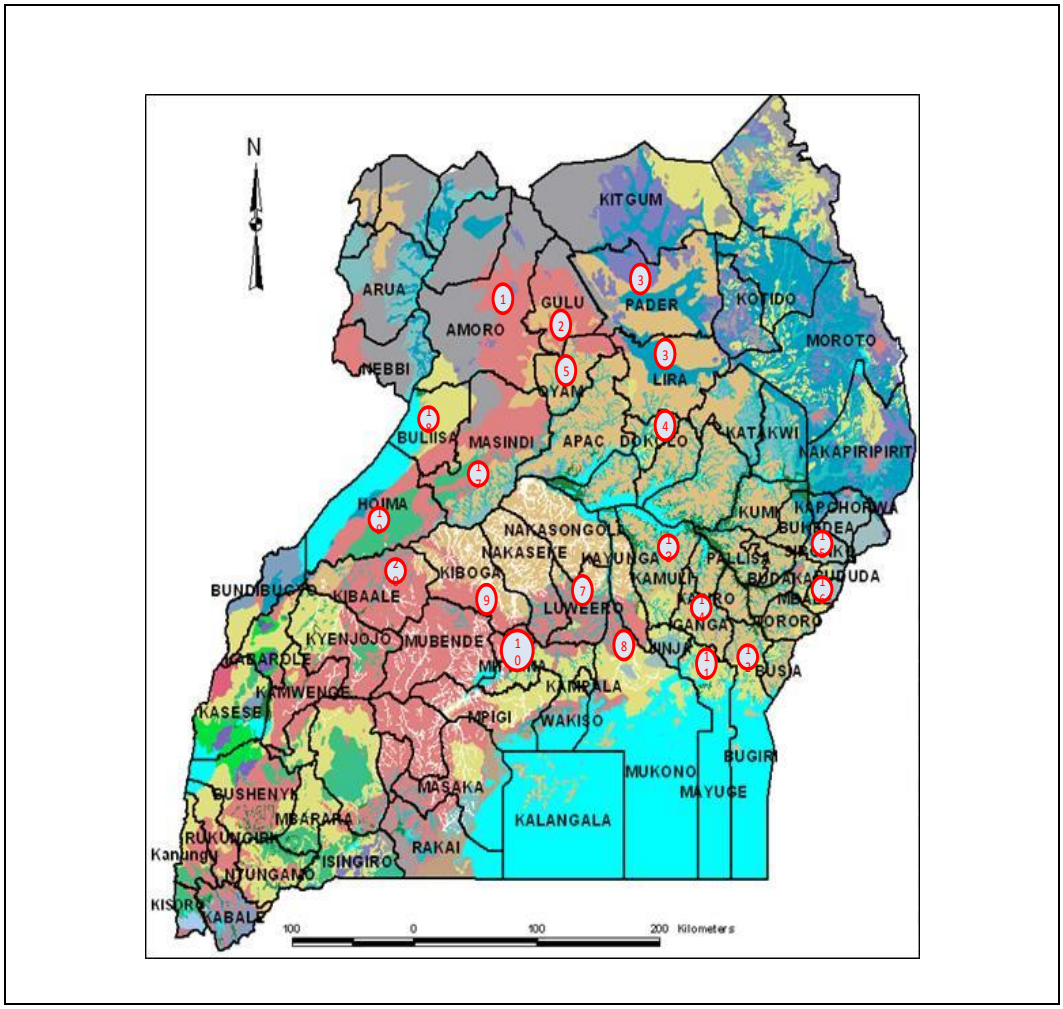

Figure 1: Map of Uganda showing districts where new banana varieties have been promoted

Identification of genes responsible for Fusarium resistance in EAHBs

This study aimed at understanding the mode of banana resistance to Fusarium basing on comparative transcriptome analysis of 'Sukalindiizi', a hybrid 'TMB2X8075' and cooking banana 'Mbwazirume' with varied response to the pathogen *Fusarium oxysporum f. sp. Cubense*. Transcriptome profiling of root samples identified 2,864,015,808 transcripts after early interaction with the disease at 0hrs, 48hrs, 96 hrs, and 192 hrs post inoculation. The raw reads were assembled to the diploid banana (*Musa acuminata ssp* Pahang) reference genome (http://banana-genome.cirad.fr/content/download-dh-pahang) using the Geneious 8.02 software. Differential gene expression (up or down regulation) was highest in the susceptible genotype 'Sukali Ndiizi' and lowest in the immune genotype 'Mbwazirume' at all time points. The genes will be annotated to identify their associated pathways and their functionality.

Developing banana populations for mapping weevil resistance

An F2 populations were generated from a diploid by diploid cross of a weevil susceptible Kasaska with a resistant Boneo (*Musa acuminata microcarpa*). In 2014, the population was used to assess weevil damage to field grown and potted plants. Bioassays of weevils on corms showed significant variation in larvae head capsule width, body weight and larva mortality, indicating segregation. In contrast, there was no segregation in the dry matter or corm hardness suggesting that the underlying plant effect on weevil is chemical rather that physical.

Data on different parameters showed that body weight, peripheral damage and total damage had the highest heritability of 33.4, 32.0 and 24.0, respectively compared to other larvae resistance and weevil damage parameters. This shows that these parameters are highly inherited and can be used for selection among in diploid breeding.

MOLECULAR BANANA BREEDING

Development of regenerable cell suspensions

Activities for establishing transformable and regenerable embryogenic cell suspensions (ECS), the starter banana material for molecular breeding improvement are ongoing. ECS of two important varieties 1) Nakitembe cell lines NKT745 and NKT 746 (Plate 2) and Gonja Nakatasanse are available for transformation. Dessert banana ECS of cultivar Sukali Ndizi and recently those of Gros Michel (Bogoya) have been established at NARL Kawanda. They are routinely subcultured by transferring into fresh medium after every 7-10 days.

Plate 2. Nakitembe cell lines NKT745 and NKT

Developing transgenic bananas for Fusarium wilt (FOC 1)resistance.

Two dessert banana cultivars Sukali Ndizi and Gros Michel (Bogoya) that are also susceptible to *Fusarium* wilt are targeted for genetic improvement under FOC subproject using RNAi constructs A, B, C, D2, and G. Regenerants of A, B, D and G are at various stages of development. There are between 200-300 shoots per construct on proliferation medium. PCR screening of shoots of proliferation medium is ongoing. 51, 29, and 52 shoots have so far tested PCR positive for A (Plate 3), B and D constructs respectively. The most advanced PCR positive lines are being weaned and acclimatized for FOC resistance screening in pot trials. Transformation of Bogoya is at initial stages of development.

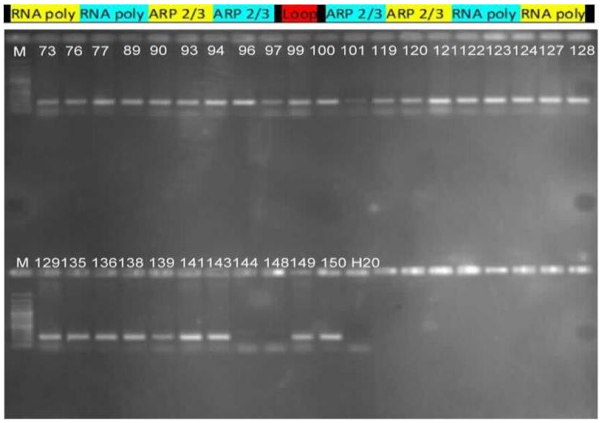

Plate 3. PCR positive Sukali Ndizi transformed with RNAi contruct A for FOC 1 resistance

Developing transgenic bananas for nematode resistance.

Data collected from the transgenic lines being evaluated in the CFT at NARL, Kawanda shows that the repellent technology as single or stacked with the rice cystatin gave over 90% resistance to the most damaging parasitic nematode, *Radopholus similis* based on the mother crop cycle data for banana cultivar Sukali Ndizi (Table 2). Promising transgenic banana lines selected from the CFT for further evaluation to determine stability of resistance to banana parasitic nematodes. The repellent gene technology is also going to be stacked with RNAi technology.

Table 2. Banana transgenic lines with nematode resistance

Transgenic line	Peptide transcription levels by qPCR	Resistance levels
OcIΔD86&peptide_line 9	+++	100% ± 0%
OcIΔD86&peptide_line 16	+++	95% ± 7%
OcIΔD86&peptide_line 11	++	99% ± 1%
Peptide_line 21	+++	94% ± 7%
Peptide Line1	+++	92% ± 7%
Peptide_line 23	++	95% ± 4%
PDI_line 38		92% ± 8%

Developing transgenic bananas for banana bacterial wilt resistance.

Following successful evaluation of BBW transgenic resistance in a CFT at NARL-Kawanda, six lines of Ndizi/ Nakyinyika immune to BBW were identified. These are being further evaluated in a multi-locational trials CFT at NARL and others to be established in Bulindi and Mbarara. The fused version (Hrap/Pflp) genes from sweet pepper, proven to work against BBW were used to transform more popular varieties of Nakitembe and Matooke Hybrid M9 (Kabana 6H). The regenerants of the transformed M9 cells are at various stages of development. There are 100-200 shoots of M9 transformed with the fused gene construct Hrap/Pflp. There are about 70 PCR positive M9 lines for either Hrap or Pflp (Plate 4) that have been weaned and are ready for inoculation. Transformation of EAHB cv Nakitembe with a stuckedconstruct Hrap/Pflp are at initial stages of development. Both M9 and Nakitembe with BBW transgenic resistance generated here are targeted for commercial release.

Plate 4. PCR positive M9 lines with Hrap and Pflp genes for resistance to BBW at weaning stage

Generating bananas with enhanced levels of provitamin A

Activities reported this year are related to the main goal of developing 800 independent lines of cultivars M9 and EAHB cv Nakitembe containing both Ubi-APsy2a (200 lines) and Aco-Apsy2a (200) of each cultivar. The idea is to select elite lines in the field of modified Nakitembe and M9 with atleast 20 μ/g dry weight of β-carotene equivalents and with no apparent yield penalty or other negative agronomic effects. A total of 300 lines of ACO-Psy2a; 522 of Ubi-Psy2a have been regenerated and established *invitro*. A total of 105 lines of M9 i.e 44 pGEN2- M (ACO-Apsy2a) and 52 pGEN2-P (Ubi-Apsy2a) were planted in a CFT at NARL-NARO on 1st August 2014. (Plate 5). The CFT is progressively being planted with new lines as they are generated.

Generation of transgenic lines for EAHB cv Nakitembe in October 2013. Transformations have been done targeting the cell lines with regeneration potential of at least 60%. Todate, there are a total of 51 shoots of Nakitembe with 36 of pGEN2-P (Ubi-Apsy2a); 7 shoots of pGEN 2-M (ACO-Psy2a) and 8 shoots of Ubi-Gus established *invitro*. The products in this activities are will also be lined for commercial release.

Plate 5. M9 with PVA enhancing genes in a CFT at NARL-Kawanda

Banana Tracker: An effective computer based system for recording and tracking all transgenic events from explant to release of elite line and recording all performance data was successfully established at NARO and is in use. All data for cell lines data, the transformation experiments, regenerated lines and molecular characterisation and lines progressed to the confined field trail is continually entered in the tracker.

BANANA BACTERIAL WILT CONTROL WITH FARMING COMMUNITIES

Banana Bacterial wilt (BBW) was first reported in 2005 in Southwestern Uganda, the region that produces more than 60% of Uganda's bananas annually (Kalyebara *et al*., 2006). BBW prevalence was kept below 5% between 2005 and 2008 (Kubiriba *et al* 2012). This was due to combined use of farmer field schools and Integrated Agricultural Research for development (IAR4D) using cultural practices focusing on the communities supported by the subcounty and district action plans. In 2010, BBW prevalence in the region increased to 34% due to incomplete and distorted information reaching the farmers; inadequate systems for surveillance of the disease and inadequate mobilization of stakeholders to control the disease. In 2012, the strategy for BBW control changed to formulating BBW control action plans focusing on the region (10 districts of the Ankole region) rather than the community (100-300 farmers) by a mix of stakeholders from the region (farmers, political leaders, extension officers and administrators). Then the action plans of districts and subcounties were designed to achieve the goal of the regional action plan, rather than to support community action plans. The overall implementation of the regional plan was spearheaded and coordinated by the regional taskforce, instituted by regional stakeholders. In August, 2012, 93.4% of the farmers in selected hotspots had over 20 infected plants in their fields. By June 2013, BBW had been controlled in over 90% and over 70% of the previously affected fields in 6 and 3 hotspots respectively.

Survey data collected from all the main banana growing regions of Uganda in November 2013 revealed that 29.1% of the banana fields still had infection in the Southwest including the Kigezi region (Table 3). BBW prevalence in Ankole alone is 13%. While BBW was effectively controlled on 60.9% of the previously affected fields in the South West, BBW was effectively controlled 87% of the previously affected controlled BBW in Ankole. This translated into

banana production recovery of 40% (Figure 2), an equivalent of US$ 64.4 million in 1 year. The same approaches of BBW control have been scaled out to Kigezi and Greater Masaka regions and results will be reported next year.

Table 3. BBW prevalence in Uganda by region as at November 2013

Region	**% of farms that still BBW**	**% of previously affected fields where BBW was controlled**
Central	56.0	36.2
East	66.7	24.1
Mid-west	54.6	28.5
S.west	29.1	60.9
Over all	**51.1**	**37.4**

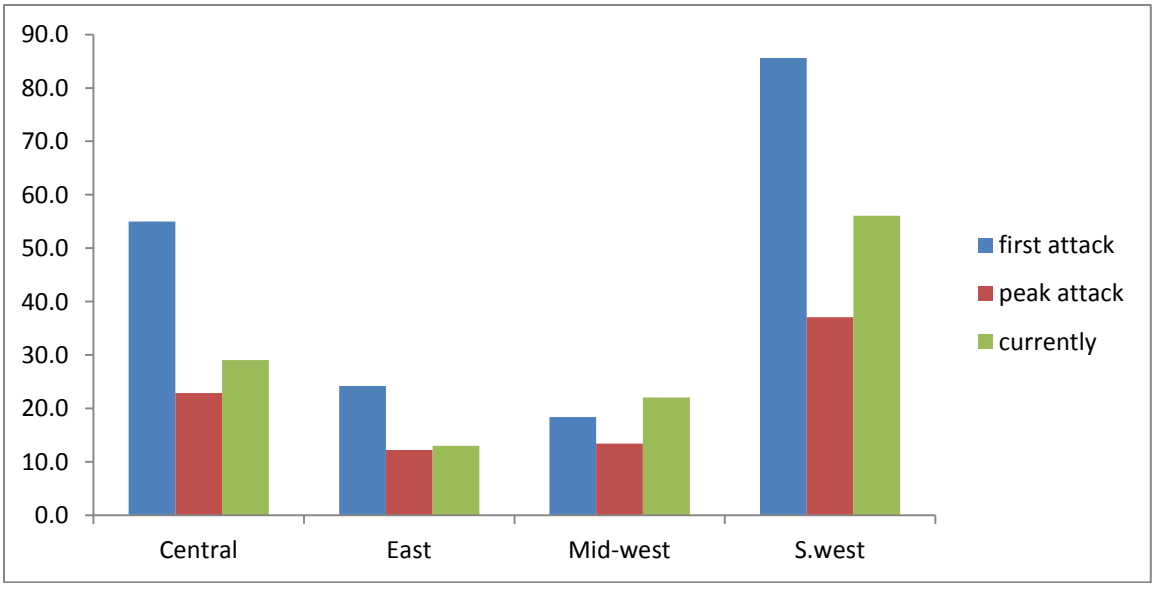

Figure 2. Banana production recovery in 4 banana growing regions in Uganda as at November 2013

Determining the carbon sequestration potential of East African highland banana cultivars

The activity was carried to assess carbon stocks of East African Highland Banana (EAHB) cultivars grown in two agro-ecological zones of Uganda- the Lake Victoria Crescent and the South-western Farmlands. Objectives were to 1) develop allometric relationships for biomass estimation of EAHB cultivars; 2) determine their carbon content; and 3) estimate their carbon stocks. Four allometric equations for cultivars *Enyeru, Nakinyika, Kibuzi_Nakitembe* were developed. It was established that banana cropping system enables much more carbon at 81-92 $Mgha^{-1}$ to be stored in the soil; hence placing banana cultivars close to, and even better than some, woody species in the Soil Organic Carbon(SOC) stock spectrum.

Conclusion

Achievements obtained this year put the National Banana Research Programme firmly on its path to developing products that will be used to establish a more productive banana cropping system in Uganda. The rate of new matooke genoptype established in EET from the breeding activities doubled and a banana population was found to segregate for weevil resistance. There are therefore more opportunities developing more matooke hybrids with both black sigatoka and weevil resistance. During this reporting period, M9 and Nakitembe transgenic lines with resistance to BBW and with enhanced PVA with products lined for commercial release were generated and are in CFT and screenhouse respectively. Finally, survey data show that BBW is largely under control in the areas of the South West that produce more than 70% of Uganda's banana.

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