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

**NARO-Bioversity Banana Improvement Project Report**

# Annual Report, January-December 2018

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## TABLE OF CONTENTS

## List of Figures



## List of Tables

# ACRONYMS

| | |
|---|---|
| AATF | African Agricultural Technology Foundation |
| ABSPII | Agricultural Biotechnology Support Project II |
| BECA-ILRI, | Biosciences East and Central Africa-International Livestock Research Institute |
| BXW | Banana Xanthomonas Wilt |
| cDNA | Complementary DNA |
| CFTs | Confined Field Trials |
| CGIAR | Consultative Group on International h |
| CIRAD | Agricultural Research Centre for International Development |
| CoEC | Codes of Ethical Conduct |
| COMESA | Common Market of East and Southern Africa |
| DG | Director General |
| DRC | Democratic Republic og Congo |
| DUS | Distinctness Uniformity and Stability |
| EAC | East African Committee |
| EAHB | East African Highland Bananas |
| ECS | Embryogenic Cell Suspensions |
| EET | Early Evaluation Trials |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EMM | Enterprise Mobility Management |
| EWEAS | Early-Warning, Early-Action |
| FERA | Food and Environment Research Agency |
| FET | Farmer Evaluation Trials |
| Foc | Fusarium oxysporum |
| FocTR4 | Fusarium oxysporum f.sp.cubense tropical race 4 |
| FTO | Freedom to Operate |
| GDP | Gross Domestic Progress |
| GMO | Genetically Modified Organism |
| H.E | His Excellence |
| HPLC | High Performance Liquid Chromatography |
| IITA | International Institute of Tropical Agriculture |
| IPR | Intellectual Property Rights |
| ITC | International Transit Centre |

| | |
|---|---|
| M&E | Monitoring and Evaluation |
| MAAIF | Ministry of Agriculture Animal Industry and Fisheries |
| MAS | Marker Assisted Selection |
| MBAZARDI | Mbarara Zonal Research and Development institute |
| MLCFTs | Multi location Confined Field Trials |
| MLTs | Multi location Field Trials |
| MSI | Millennium Science Initiative |
| NABIOs | NARO-Bioversity hybrids |
| NACoRI | National Coffee Research Institute |
| NACRRI | National Crop Resources Research Institute |
| NARL | National Agriculture Research Laboratories |
| NARO | National Agricultural Research Organisation |
| NARS | National Agriculture Research Systems |
| NaSAARI | National Semi-arid and Arid Research Institute |
| NBC | National Biosafety Committee. |
| NBRP | National Banana Research Program |
| NPA | National Planning Authority |
| OIC | Organisation of Islamic Cooperation |
| PCR | Polymerase Chain Reaction |
| PVA | Pro-vitamin A |
| PYT | Preliminary Yield Trials |
| qPCR | Quantitative Polymerase Chain Reaction |
| RNA | Ribonucleic acid |
| RNAi | Ribonucleic acid interferance |
| RNA-Seq | RNA sequencing |
| SCIFODE | Science Foundation for Livelihoods and Development |
| SDGs | Sustainable Development Goals |
| SOPs | Standard Operating Procedures |
| SSR | Simple Sequence Repeats |
| UNBS | Uganda National Bureau of Standards |

# 1. EXECUTIVE SUMMARY

The Government of Uganda seeks to harness the agriculture sector to maximize returns to the economy and achieve the country's Vision 2040 of transforming Ugandan society from a peasant to a modern and prosperous country. In the agriculture sector, investment between 2015-2020 was planned to focus on 12 crop enterprises including bananas. Annual banana output is estimated at US$ 550 million, an equivalent of 10.4% of Agriculture sector's target contribution (US$5.3billion) to Uganda's GDP projected for Vision 2040 as at 2017. This contribution is significant even when banana's average productivity on farm is just 10t/ha/yr versus the crop's potential of over 60-70ton ha-1 yr-1 (Van Asten et al., 2005). Low productivity is attributed to poor production techniques, over dependency on rain fed agriculture, poor management of pests and diseases; limited application of technology and innovation among others. Uganda's national biotechnology strategy, championed by this project, to complement other approaches to increase productivity of bananas and other crops is envisioned to play a key role in the realisation of Vision 2040. Hence the major objectives of the project were: i). to build human and infrastructural capacity needed to subtend the use of biotechnology innovations as they become available and to access the technologies for safe application. In this regard, NARO scientists have acquired critical skills for generating transgenic plants routinely and advancing biotechnological approaches, including generation of embryonic cell suspensions and transformation, bioinformatics and gene cloning. Over the last 5 years, the project has trained/facilitated training of 11 PhDs, 13 at MSc and a lot more technicians on specific skills. These have taken responsibilities across the country. Furthermore, the project team has been a core source of human capacity for other biotechnology initiatives in other NARO institutes such as NaCRRI, NaSARRI, KaZARDI and NaCORI. In terms of infrastructure, the project has provided and maintained a back-up power generator to ensure 24/7 power supply; tissue culture to facilitate micropropagation, banana cell and embryo cultures, transformation and regeneration of transgenic lines; and, a biosafety level II (BSLII) fully equipped Biotech/Molecular laboratory buildings. ii). Effective transfer of technologies – to ensure that the country achieves ownership of the technology with the tools and approaches transferable to other important crops. The development of regenerable cell suspension technology for East African Highland bananas has been perfected at NARO-Uganda. Cell suspensions of Sukali Ndizi,

Gonja and Nakitembe for engineering weevil/nematode and BXW resistance, PVA into local landrace bananas have been developed by the project and currently, Nakitembe cell lines (741, 743, 745 and 747) have been cryopreserved.

The project has also accessed and developed two genes for the management of weevils and nematodes- *Carica papaya* cystatin genes (*CpCYST*) engineered into Gonja and Nakitembe for nematode resistance and *Bacillus thuringiensis* crystalline (*Cry6A*) genes engineered in Gonja and Nakitembe clones of EAHB for weevil resistance. The two genes (*Cry6A* and *CpCYST*) were cloned into a stacked construct, which increased mortality by 8.7% in weevils over the individual genes.

For the first time in Uganda, resistance genes have been isolated internally (by the project), a development that will allow the development and deployment of locally patented genes with full freedom to operate (FTO) powers. Foc race 1 resistance genes from Mbwazirume (AAA-EA) were isolated using Next Generation Sequencing (RNA-Seq data)and transcriptome analysis and are currently undergoing Allergenicity and Toxicity data analyses. In terms of molecular characterisation, the project is developing capacity for gene-insert analysis, transcript and protein expression analysis to generate information for deregulation and release of biotech products.

The Project Steering Committee (2009) made a turning-point recommendation to integrate genetic engineering and conventional breeding tools, exploiting synergies between the two sets of tools to enhance the development of new and stress-resistant genotypes. This meant that multi-trait resistance could be introgressed into priority, preferred but susceptible land races and strengthened by genetic engineering killer disease resistance genes. This also necessitated the development of markers to enhance marker-assisted selection. In this regard access to a properly characterised Musa collection is a pre-requisite and up to 500 accessions have been assembled at MBAZARDI in southwestern Uganda. In this direction, hybrids (the NARO-Bioversity hybrids, NABIOs for short) were developed for their resistance primarily to black sigatoka (but also for weevils and nematodes). Cell suspension of four of the NABIOs are currently being developed for transformation with BXW-resistance genes (*hrap* and *pflp*) to control BXW to which all cultivated genotypes succumb. The conventionally bred NABIOs will be released in August 2018.

Uganda's support to banana biotech work is already bearing fruits in all fronts and with more support, the project will service other NARO biotech initiatives and better contribute the national strategy for harnessing biotechnology. The products from these projects together will increase productivity of banana cropping systems by over US$ 100 million annually, protect over 30% of children and reproductive women from vitamin A deficiency and increase plantation longevity for more than 35 years across central, mid-western and eastern regions of Uganda, where, over the last 5 decades, banana production has experienced decline. The advancement of these products to commercial release will clear the way for products of other NARO's biotech initiatives.

# 1 WHY BIOTECHNOLOGY AND GENETIC ENGINEERING FOR UGANDA?

Agriculture remains a core sector of Uganda's economic growth. It is one of the strategic opportunities that the Government of Uganda seeks to harness to maximize returns to the economy to achieve the country's Vision 2040 of transforming Ugandan society from a peasant to a modern and prosperous economy in 30 years. However, the sector is growing at a sluggish rate of 1.5% annual compared with a population growth of 3.5%. This means Uganda will ultimately be a net food importer unless new strategies are developed to significantly increase productivity across the sector. Moreover, under the current trends of climate change phenomenon, the country is experiences prolonged draughts, high temperatures, short but intense rainfall leading to floods, upsurges of pests and diseases, all resulting in frequent crop failures and declining environmental quality. These factors are likely to render economic transformation with agriculture playing a key role very difficult unless non-conventional but scientific innovations are adopted. It is widely believed that the major transformation will however be achieved with significant shift of labour force from agriculture to industry and service sectors (Figure 2.1). This implies that the productivity for land and the remaining labour force in the agriculture sector must correspondingly increase to match the increased demand for agricultural products for the budding agro-processing industry.

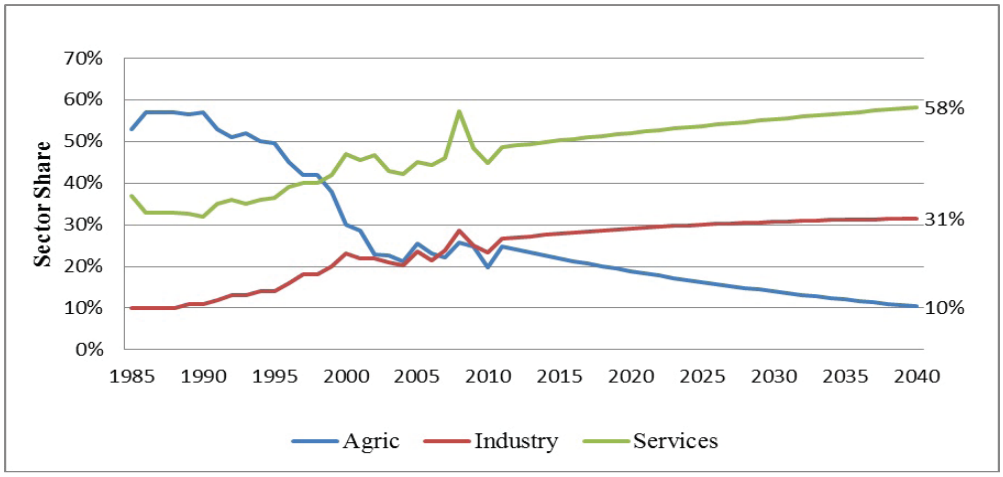


**Figure 2:1. Shift of labour force from agriculture sector to industry and services sectors**
Source: National Development Authority, 2010

## 1.1 The role of bananas

Banana is an important food and income crop for millions of smallholder farmers in Africa. Farming communities in Uganda have consistently ranked the banana crop as number one. The crop ranks first in terms of area occupied, total production and per capita consumption in Uganda. It is grown by over 75% of smallholder farmers owing to its unique advantages of producing acceptable yields amidst erratic rainfall, and a perennial nature coupled with an all-year-round fruiting character. These attributes characterize banana as an ideal crop for household food, nutrition and income security. The 5 year National Development Plan 2015-2020, which aims at increasing per capita income from US$788 to US$1,039 (middle income status); prioritizes investment in agriculture; tourism and minerals, oil and gas. In agriculture, investment will focus on 12 enterprises; cassava, maize, groundnuts, fisheries, livestock, bananas. Cooking bananas (Matooke) alone account for 80% of the annual output and is an estimated value of US$440 million using average farmgate price (Kalyebara et al., 2006). This is about 8.3% of Agriculture's target contribution (US$5.3billion) to Uganda's GDP projected for vision 2040 as at 2017. Banana is the main staple food and cash crop for more than 20 million smallholder farming and trading Ugandans. Over the last five decades, banana provided 3-22% of total calorie consumption per capita, which is estimated at 147 kcal per person. Banana, therefore, will no doubt be among the key drivers of Uganda's development agenda as source of food, income and employment.

Productivity of most crops, banana inclusive, has been reducing over the last decade. Banana average yields do not exceed 30% of the crop's potential of over 60-70 ton ha-1 yr-1 (Van Asten et al., 2005). This is attributed to poor production techniques, over de-pendency on rain fed agriculture, poor management of pests and diseases; limited application of technology and innovation among others. Specifically, banana production is constrained by a complex of biotic (banana Xanthomonas wilt or BXW, weevils, nematodes, black Sigatoka) and abiotic stresses (nutrient deficiencies and drought stress).

Farmers manage some constraints through cultural control, many of which are effective in keeping the pest and disease pressures below threshold levels. For example, disruption of the banana weevil's life cycle by destruction of the breeding sites drastically reduces the weevil population and damage. Data collected on-farm for three years showed that banana weevil population could be reduced from 52,000 to 13,000/ha, with associated reduced corm damage of 41% and yield increase of 70% (Masanza et al., 2003). BXW prevalence reduced from about 45% in June 2012 to about 13% in September 2013, with banana production recovery of 40% from the peak of BXW epidemic in all the 10 districts of the major banana growing region of Ankole (Kubiriba et al., 2016). Cultural control involves manipulation of environment of the plant host and the parasite. It is a continuous and tedious process for the farmers and the supportive development machinery and can only work in the short run. Diseases such as Fusarium wilt are not controllable by cultural practices. Use of resistance is more effective and durable option for the management of banana problems. Application of this conventional breeding approach has been hampered by high sterility of the major preferred cultivars, polyploidy, parthenocarpy and a long cropping cycle. Use of biotechnological tools may compliment efforts conventional breeding in improving bananas.

According to the International Service for the acquisition of agri-biotech applications (ISAAA) report of 2016, the market value of GM crops was US$15.8 billion globally, an equivalent of 35% of the global commercial seed market (US$45 billion). In 2015, the total benefits were US$15.4billion comprising of a 15% reduced production cost and 85% yield gain. Between 1996 and 2015, the 574million tons of productivity gained saved 174 million hectares of land from being ploughed. GMO technology has,

additionally, reduced chemical pesticide applications by 37%. If the global rate of biotech crop planting matched that of the US, global greenhouse missions would fall by 0.2 billion tons of $CO_2$ and would allow 0.8 million hectares of cropland to be converted back to forests and pastures. In a Forum on the Future of Agriculture, the FAO Director General Jose Graziano da Silva underscored the need to utilize a broad portfolio of tools and approaches, including biotechnology to eradicate hunger, malnutrition and achieve sustainable agriculture.

## 1.2 Uganda adopts biotechnology.

After careful consideration, NARO-Uganda decided to address banana production constraints through biotechnology and to develop national biotechnological capacity largely because- i). cultivated bananas are essentially sterile and thus, environmental concerns that affect other crops, regarding the possibility of 'gene flow' to weeds, local crops, or wild relatives of crops, are negligible; ii). the genes immediately available for engineering resistant bananas are derived primarily from banana wild relatives, though initially for training purposes common food crops such as rice and maize that are already readily consumed in the country would be used; iii). the East African Highland Banana (EAHB) and other 'staple food' bananas, though of vital importance regionally, have been neglected by the international private sector and research organizations. Thus, NARO would not be in competition with better-resourced organizations but instead took the opportunity to gain leadership in developing this crop.

In the early 2000s, Uganda adopted biotechnological approaches as a strategy to address agricultural productivity problems and as a complementary measure to already existing crop improvement conventional methods. Uganda Government decided to become a member of the Consultative Group on International Agricultural Research (CGIAR) and negotiated use of its membership contribution to build capacity in modern biotechnology. 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. It is on this basis that the project: *Novel Approaches to the Improvement of Banana Production in Eastern Africa - the application of biotechnological methodologies* was formulated. The capacity would be acquired in an apprenticeship mode where experts in modern biotechnology would backstop Ugandan scientists to address critical

agricultural challenges as they acquired the skills. It was envisaged that the modern biotechnological approach would complement the already existing conventional methods. Bioversity International (then known as the Institute of Plant Genetic Resources) through its department of International Network for Improvement of Banana and Plantain (INIBAP), was tasked to coordinate the partnerships required to deliver the capacity and associated biotechnology products.

## 1.3 Project objectives

The overall objective was to develop East African Highland bananas with resistance to pest and disease constraints (weevils, nematodes, black Sigatoka and Fusarium wilt) using novel biotechnological approaches. The specific objectives were:

- **Capacity building** – to ensure that a cadre of experts and critical infrastructure are made available to support the research and development of innovations as they become available and to apply those of most value 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
    - Genetic transformation systems and tools for EAHBs internalized
    - Genes from technology partners accessed and evaluated
    - Important genes for improvement of banana isolated cloned and evaluated
- **Partnerships and Networking** – at the global level to draw in cutting edge science and technological advancement; at the national level to facilitate technology transfer to other crops;
- **Effective governance -** to ensure that resources are invested only on priority constraints; that the project is regularly reviewed and corrective measures take in time; and, research progress monitored and information shared;

Subsequently other issues emerged as important constraints - drought, micronutrient deficiencies, delayed ripening and plant architecture. In addition, the project made some strategic decisions to include banana germplasm conservation (key to genetic variability) and characterization and to integrate genetic engineering and cross-

breeding tools to exploit the synergies between the two approaches. The choice of banana as a means of developing national biotechnological capacity was based on:

a) 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.
b) The 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).
c) No other organizations (private or public) were focusing their research on East African Highland bananas (EAHB), the bananas that are unique to east and central Africa; hence the research would not compete with better-resourced organizations.

# 2 BUILDING CAPACITY FOR GENETIC ENGINEERING OF CROPS.

## 2.1 Human resource capacity

In the early 2000s, NARO did not have critical infrastructure and employed very few skilled scientists and technicians trained in biotechnological tools to address the priority constraints of agriculture in Uganda. As such, capacity building was required for effective research and development of innovations and the project emphasized extensive training of NARO scientists and technicians. The project outputs have primarily benefitted the people of Uganda through human capacity training like short-term courses, MSc and PhD. For example, over 10 scientists have been trained at MSc and PhD levels (Table 3.1). Skills include generation of embryonic cell suspensions, transformation and characterization for confirming presence of genes, their insertion and expression in the transgenic materials. Additionally, a few staff have skills in bioinformatics and gene cloning, all essential for advancing biotechnological approaches. With the skills acquired, these scientists are now an integral part of teams generating genetically modified plants, with expertise in developing embryogenic cell suspensions for East African Highland bananas (first successfully done at NARO-Uganda) and development of gene constructs and engineering them into plants/crops of interest. As a result, transformation and molecular characterization of transgenic plants are now routinely carried out at the National Agricultural Research Laboratories (NARL), NARO-Uganda. Increased advancement in the application of biotechnology has seen some staff undergoing training and obtaining additional skills in bioinformatics, cryopreservation and gene cloning skills (Table 3.1).

Horizontally, the banana biotechnology team has been a core source of human capacity for other biotechnology initiatives in other NARO institutes such as National Crop Resources Research Institute (NaCRRI), National Semi-Arid Resources Research Institute (NaSARRI), Kabale Zonal Agricultural Research and Development Institute (KAZARDI), National Coffee Research Institute (NaCORI) and private tissue culture laboratories (Figure 3.1). Some staff were directly hired by universities such as Kyambogo University, Makerere University and Gulu University while others received training at NARL. Students from tertiary institutions have also tapped into the project activities to build capacity through practical short training (internships) and project

research. This is healthy development and fulfilment of the technology transfer objective across NARO and other agricultural institutes and which merits continuous support.

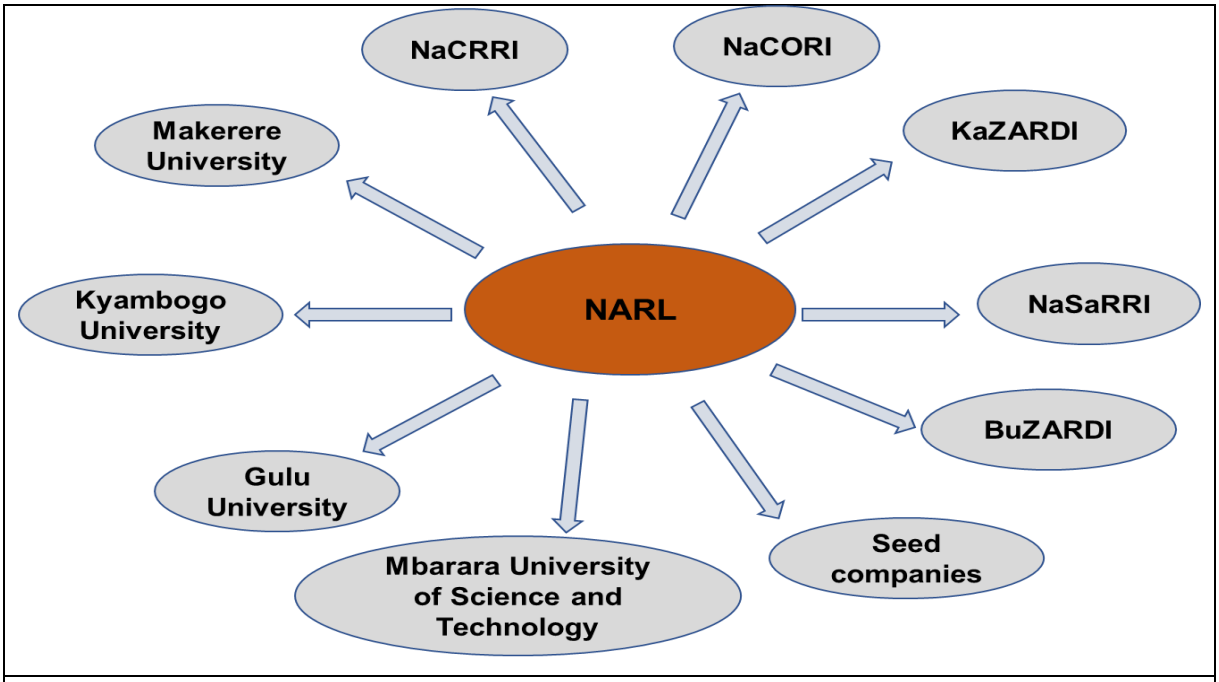


**Figure 3:1.** Building capacity for genetic engineering of crops in Uganda

**Table 3.1.** List of scientists trained in various biotech skills

| Name | Level | Current position | skill | Application | Current placement |
|---|---|---|---|---|---|
| Geoffrey Arinaitwe | PhD | Program Leader, BB & PGR | Transformation | Regenerate transgenic plants, Stewardship and regulation, field trial management | NARO-NARL |
| Abel Sefasi | PhD | Lecturer | Transformation | Regenerate transgenic plants, Molecular characterisation | University of Blantyre, Malawi |
| Betty Magambo | MSc | Research Officer | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | NARO-NaCORI |
| Clara Samukoya | MSc | Research Assistant | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | NARO-NaCRRI |
| Eliezar Bakazze | MSc | PhD student, RO | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | NARO-NARL |
| Annet Namuddu | MSc | PhD student | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | NARO-NaCRRI |
| Richard Echodu | PhD | Lecturer | Transformation | Regenerate transgenic plants, Molecular characterisation | Gulu University |
| Georgina Mwaka | MSc | PhD student | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | Stellenbosch University- RSA |
| Rebecca Nakacwa | MSc | Private sector | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | - |
| Caesar Oweitu | BSc | MSc student | Transformation | Regenerate transgenic plants, Molecular and phenotypic characterisation | NARO-NARL |
| Andrew Ssenyonjo | BSc | Research Assistant | Transformation | Regenerate transgenic plants | CIP-Uganda |
| James Kawuma | BSc | Research Assistant | Transformation | Regenerate transgenic plants | CIP-Uganda |

| | | | | | |
|---|---|---|---|---|---|
| Priver Namanya | PhD | Research officer | Transformation, Tissue and cell culture | Embryo cell suspensions; Cryopreservation, Molecular characterisation, Stewardship and regulation, field trial management | NARO-NARL |
| David Talengela | PhD | Research Scientist | Transformation, Gene isolation and cloning | Gene construct design, Molecular and phenotypic, characterisation of transgenics, Stewardship and regulation | |
| Abubaker Muwonge | PhD | Research officer | Transformation, Gene isolation, cloning and bioinformatics | Gene construct design, Molecular, phenotypic characterisation of transgenics, Stewardship and regulation | NARO-NaCRRI |
| Jimmy Tindamanyire | MSc | Leader, Bioversity Project | Transformation, Gene isolation and cloning | Gene construct design, Molecular characterisation, Stewardship and regulation, field trial management | NARO-NARL |
| Henry Mwaka | MSc | PhD student | Transformation, Gene isolation and cloning | Gene construct design, Molecular characterisation, Stewardship and regulation | NARO-NARL |
| Andrew Kiggundu | PhD | Cassava Project Manager | Gene isolation, cloning and bioinformatics | Gene construct design, Molecular characterisation of transgenics, Stewardship and regulation, field trial management | Donald Danforth Center- USA |
| Stephen Buah | PhD | Research officer | Gene isolation and cloning | Gene construct design, Molecular characterisation of transgenics, Stewardship and regulation, field trial management | NARO-NARL |
| Francis Onyilo | MSc | PhD student | Gene isolation and cloning | Gene construct design, Molecular characterisation | NARO-NARL |
| Ssekiwoko Fred | PhD | Research officer | Disease resistance | Disease diagnostics, Molecular characterisation | NARO- Bulindi |
| Kubiriba Jerome | - | Senior Research Officer | Disease resistance | Disease diagnostics, Molecular characterisation, biosafety, stewardship and regulation, field trial management | NARO-NARL |
| Henry Buregyeya | MSc | PhD Student | Disease resistance | Disease diagnostics, Molecular characterisation, Nutritional analysis | Makerere University-Uganda |

| | | | | | |
|---|---|---|---|---|---|
| David K. Okello | PhD | Research officer | Disease resistance | Disease diagnostics, Molecular and phenotypic characterisation, field trial management | NARO-NaSARI |
| Reuben SSali Tendo | PhD | Research Associate | Disease resistance | Disease diagnostics, Molecular characterisation, field trial management | CIP- Uganda |
| John Adriko | PhD | Research officer | Disease resistance | Disease diagnostics, Molecular characterisation | NARO-NARL |
| Ivan K. Arinaitwe | MSc | PhD student | Disease resistance, Tissue and cell culture | Micropropagation, Disease diagnostics, Molecular characterisation, field trial management | Malaya University-Malaysia |
| Abel Arinaitwe | MSc | PhD student | Disease resistance | Micropropagation of transgenic plants for CFT; Disease diagnostics, Stewardship and regulation, field trial management | NARO-KaZARDI |
| Kenneth Ssekatawa | MSc | Biology Teacher | Pest resistance | Disease diagnostics, Molecular characterisation | Gayaza High School |
| Ruth Mbabazi | PhD | Post-Doc Fellow | Biosafety regulation | Nutritional analysis, Deregulation of GMs for food, Stewardship and regulation, field trial management | Michigan State University- USA |
| Moses Matovu | PhD | Research officer | Food standards and safety | Nutritional analysis | NARO-NARL |
| Naboth Oyesigire | - | Research Assistant | Tissue and cell culture | Micropropagation of transgenic plants for CFT; bulking conventional breeding materials for on-farm testing, Stewardship and regulation, field trial management | NARO-NARL |
| Jane Arinaitwe | - | Research Technician | Tissue and cell culture | Micropropagation of conventional breeding materials for on-farm testing | NARO-NARL |
| Pamela Lamwaka | BSc | Research Assistant | Tissue and cell culture | Embryo cell suspensions; Cryopreservation | NARO-NARL |
| Loice Natukunda | PhD | Lecturer | Tissue and cell culture | Embryo cell suspensions; Cryopreservation | UTAMU-Uganda |
| Henry Basheija | MSc | IT Manager | Tissue and cell culture | Embryo cell suspensions; Cryopreservation | NARO-NARL |
| Doreen Amumpaire | MSc | Research Assistant | Tissue and cell culture | Embryo cell suspensions; Cryopreservation, Stewardship and regulation | NARO-NARL |

## 2.2 Infrastructural capacity

### 2.2.1 Laboratory space

To match the quality of research and development at NARL, the project has focused on development, procurement and continuous maintenance of critical infrastructure (buildings, laboratory consumables, equipment, screenhouses, confined field trials and germplasm). The practical application of genetic engineering is largely carried out in two spaces (Tissue Culture and Biotech/Molecular buildings respectively) which have been classified as biosafety level II (BSLII) facilities.

The Biotech/Molecular Lab facility is stocked with equipment (for example, PCR and QPCR machines, electrophoresis systems, -20 and -80 °C freezers, HPLC machine, freeze-dryer, fume hoods, Southern hybaid ovens, NanoDrop system) used in biochemical and genetic analyses. Such analyses include, PCR, Southern blot hybridisation, extraction and analysis of nucleic acids, gene discovery and high-performance liquid chromatography. The Tissue Culture Lab facilitates micropropagation, banana cell and embryo culture, transformation and regeneration of transgenic lines and is equipped with autoclaves, laminar flow hoods, water de-ioniser system and growth cabinets and chambers.

Over the years, the high volume of biotechnology activities and different research teams has overwhelmed these facilities. Therefore, the project prioritised the need to expand laboratory space and procure additional new equipment to meet current and future demand. Consequently, the project constructed an extension to the tissue culture laboratory (with two transfer rooms, two growth rooms and a store) (Figure 3.2) at NARL, Kawanda. The laboratories are also supported by a back-up power generator to ensure that the laboratories remain functional in the face of possible power cuts on the national grid. 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 has planned for construction of a new Tissue Culture facilitate (Figure 3.2).

### 2.2.2 Greenhouse and Screenhouse space

Once putative transgenic lines are confirmed positive with transgene, they are bulked and weaned off in humid chambers located in the greenhouse. Through partnerships and collaborative work, the Agricultural Biotechnology Support Project II (ABSPII) Project supported development of the Greenhouse facilities at NARO-NARL. Once weaned plantlets harden off, they are transferred to pots before they are screened for resistance to respective biotic and abiotic stresses.

**Figure 3:2.** Artistic impressions of the new Tissue culture laboratory

Prior to confined field trials, preliminary screening of generated transgenic plants is performed under controlled conditions in screenhouse. Upon completion of pot experiments in the screenhouse, transgenic lines of interest are selected and advanced for CFT studies. To facilitate this, two biosafety (BSLII) screenhouses of 60 $m^2$ (with four compartments) each have been constructed. The first screen house space facilitates screening of banana for resistance to fusarium wilt while the second is used in screening for BXW resistance.

However, the 120 $m^2$ area is not sufficient enough to take care of the increased number of transgenic lines from different Projects. This therefore necessitated refurbishing an existing structure for screening transgenic banana lines (Bioversity Project) for resistance to nematodes and weevils. This is however a short-term solution to limited screen house space and therefore, construction of a third screenhouse is needed.

### 2.2.3 Confined field trial capacity

The application of genetic engineering in the improvement of crops is a sequential process starting with gene discovery, transformation and regeneration of transgenic lines. The products of genetic engineering (transgenic lines) are assessed under confined field trials (CFTs) and finally multi-locational field trials (MLTs) conditions. At NARO-NARL, the National Banana Research Programme is now routinely conducting CFTs and this has led to improved infrastructural and human capacity in the lead up to product development.

In the quest to realize food security and improvement of small holder farmer livelihoods in Uganda, genetic engineering has been utilized to generate transgenic cooking banana varieties with resistance to black sigatoka, bacterial wilt, weevils and nematodes while some have been biofortified with pro-vitamins. Therefore, transgenic lines have been tested under screen house conditions and promising lines tested in CFT. For example, the NARO-Bioversity Biotechnology Project set up the first ever CFT in Uganda to assess resistance to black Sigatoka in transgenic Cavendish banana. This also opened opportunities to develop human capacity regarding stewardship and biosafety regulation which has complemented subsequent and successful CFT applications to the National Biosafety Committee.

Through increased partnerships and human capacity, other banana production constraints like banana bacterial wilt (BXW) have been addressed by use of anti-BXW transgenes (ferredoxin-like amphipathic protein, *pflp* and hypersensitive response-assisting protein (*hrap*), isolated from sweet pepper (*Capsicum annuum)* and have subsequently been tested in transgenic banana under CFT conditions (Ref: GM/CFT/003/09). This activity utilized cell lines from the NARO-Bioversity biotechnology project.

The problem of destructive banana pests such weevils and nematodes has been addressed by using modified papaya cystatin (*CpCYSΔ89*) and *Bacillus thuringiensis* version Bt Crystal6A (*Cry6A*), constituted as single or stacked gene construct (s). The proof of concept was tested in Sukali Ndizi with selected lines assessed under CFT conditions (Figure 3.3). These gene constructs have been transformed into consumer preferred banana cultivars. Efficacy of these transgenes against nematodes and weevils has been evaluated in pot experiments under screen house conditions and promising transgenic lines have been selected for CFT evaluation.

Additionally, genetic engineering has been utilized in biofortification to address the problem of micronutrient deficiency in Uganda communities that heavily depend on banana as a starchy staple food. Transgenic hybrid (M9) and EAHB cultivar ‘Nakitembe’ with a banana phytoene synthase gene have been developed and assessed in CFT for pro-vitamin A (PVA) enhancement. As a step towards product development, elite transgenic lines meeting the target PVA levels and other selection criteria have been selected from the CFT and are currently being multiplied for multi-

location field trial (MLT). Again, the biofortification study utilized cell lines from the NARO-Bioversity biotechnology project.

**Figure 3:3.** CFT planted with Sukali ndizi transformed with *CpCyst* and *Cry6A*

## 2.3 Major Equipment

In addition to constructing, the laboratory buildings have been stocked with equipment important in genetic, molecular and biochemical analyses. Such include, QPCR system (purchased by MSI project) and used in many applications such as trans(gene) expression and microRNA analyses, from isolation through discovery, profiling, quantification, validation and functional analysis. Upon regeneration of putatively transgenic plants, integration of transgene is confirmed by use of PCR analysis while transgene copy numbers and profile in the transgenic plants is confirmed by Southern blot analysis using Southern hybridization ovens (funded by ABSPII Project). With increasing collaborative research, the Biofortification Project purchased a high-performance liquid chromatography (HPLC) system which is currently in use by both Biofortification and Bioversity Projects. HPLC is currently used for biochemical analysis of carotenoids in PVA enhanced transgenic banana and more recently diploids rich in PVA. Other critical equipment such as -20 and -80 °C freezers are important in gene discovery and long-term storage of consumables that require ultra-low temperatures.

One big problem regarding the equipment is cost of repair, where manpower for even minor repair of an HPLC or QPCR system would have to be flown in from as far as South Africa which makes maintenance costly. That capacity needs to be built in the country. Over last 13 years, these facilities have been overwhelmed by high volume of biotech activities and tens of scientists and technicians using them. There is need for expanding laboratory space with more new equipment to meet the current and future levels of operation. The solution to this would require building technical capacity which can also be utilized by other NARO institutes and partners.

## 2.4 Partnerships for genetic engineering of crops

### 2.4.1 National and regional level partnerships

The NARO-Bioversity partnership has attracted collaboration from a number of public sector research organizations seeking to use the capacity at National Agricultural Research Laboratories (NARL). Not only does the development of infrastructural facilities support project activities but also other collaborators at NARL. Additionally, other NARO programs on bean, cassava, coffee, groundnut, maize, potato and sweet potato have benefited from trainings and facilities at NARL biotech laboratory. This has consequently developed and nurtured technical linkages with CGIAR centres (like CIAT, CIP, IITA), regional and international research groups which has facilitated access and exchange of knowledge, materials and technologies. The project has also attracted private and public entities at regional and international level. These new players bring extra resources (human and financial) and technological know-how, which enable NARO-Uganda to advance rapidly on the agreed research agenda. For example, ABSPII, MSI and QUT partnerships who have in turn aided procurement of specialized equipment and facilities. These partners include other public agricultural researches institutes (PARIs), universities and private companies.

#### International partnerships

These include research partners like Centre De Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), France; Katholieke Universiteit Leuven (KU-Leuven), Belgium; the John Innes Centre; the University of Leeds in UK; the International Institute of Tropical Agriculture (IITA), Nigeria; the Forestry and Agricultural Biotechnology Institute, University of Pretoria (FABI), South Africa; Queensland University of Technology (QUT) of Australia, University of California, San Diego (USA) and University of Georgia, Athens (USA) as well as other development partners: the Rockefeller Foundation, USAID and the Belgium Government. Moreover, the project's impressive progress has attracted new development partners: the Rockefeller Foundation, The Bill and Melinda Gates Foundation, USAID and the Belgium Government. These development partners have provided - technological know-how and financial resources, which has enabled the project to advance rapidly on the agreed research agenda. Consequently, more

products, such as development of transgenic M09 (Matooke hybrid) with increased provitamin A (PVA) and BXW resistance in collaboration with University of Queensland, Australia and International Institute of Tropical Agriculture (IITA) respectively is underway.

# 3 DEVELOPMENT OF EMBRYOGENIC CELL SUSPENSION AND TRANSFORMATION SYSTEMS

The capacity building activities resulted into two very early benefits for NARO's biotechnology development initiatives. An embryogenic cell suspension and transformation systems for East African highland bananas were developed.

## 3.1 Embryogenic cell suspension

Using immature male buds as explants, four *Musa* spp. AAA-EA cooking banana cultivars ('Nakyetengu', 'Namwezi', 'Kisansa' and 'Nakitembe' were screened for their ability to produce embryogenic cells under *in vitro* conditions in the tissue culture laboratory. Bracts were removed from the male buds exposing immature flowers (hands) which were aseptically isolated from positions 8 to 15 under a binocular microscope in the laminar flow hood. The flowers were inoculated on Murashige and Skoog (MS)-based callus induction medium optimized for Musa AAA-EA (Namanya *et al.,* 2004) and maintained in the dark and monitored for 4 - 6 months for embryogenic callus induction. The embryogenic callus was cultured in MS salts and vitamins rich liquid (MA2) medium (Côte *et al*., 1996) while shaking in the dark.

To determine the embryogenic potential of the cell suspension cultures, samples from the suspension cultures were periodically plated on regeneration medium MA3 (Côte *et al*., 1996), maintained in the dark at room temperature and monitored for embryo development. Somatic embryos from cell suspension cultures were transferred to germination medium MA4 (Escalant *et al*., 1994) on which embryo germination was determined as the number of embryos with either shoot or root and shoot development. Germinated embryos were subsequently transferred to hormone-free MS medium on which plant development (root and shoot elongation) was achieved, followed by weaning of selected plantlets in the greenhouse (Figure 4.1).

Upon generation of cell suspension cultures, periodic plating confirmed their embryogenic potential. Within Phase I, the project successfully established two cell lines, one on Sukali Ndizi (AAB) and another on the highland banana cultivar 'Nakinyika' (AAA). This break-through opened the way for regular use of embryogenic cell suspension for the engineering of desired genes into the target cultivars. In 2013

and 2014, eight embryogenic cell suspensions of a highland banana ‘Nakitembe’ and two of plantain ‘Gonja’ were generated.

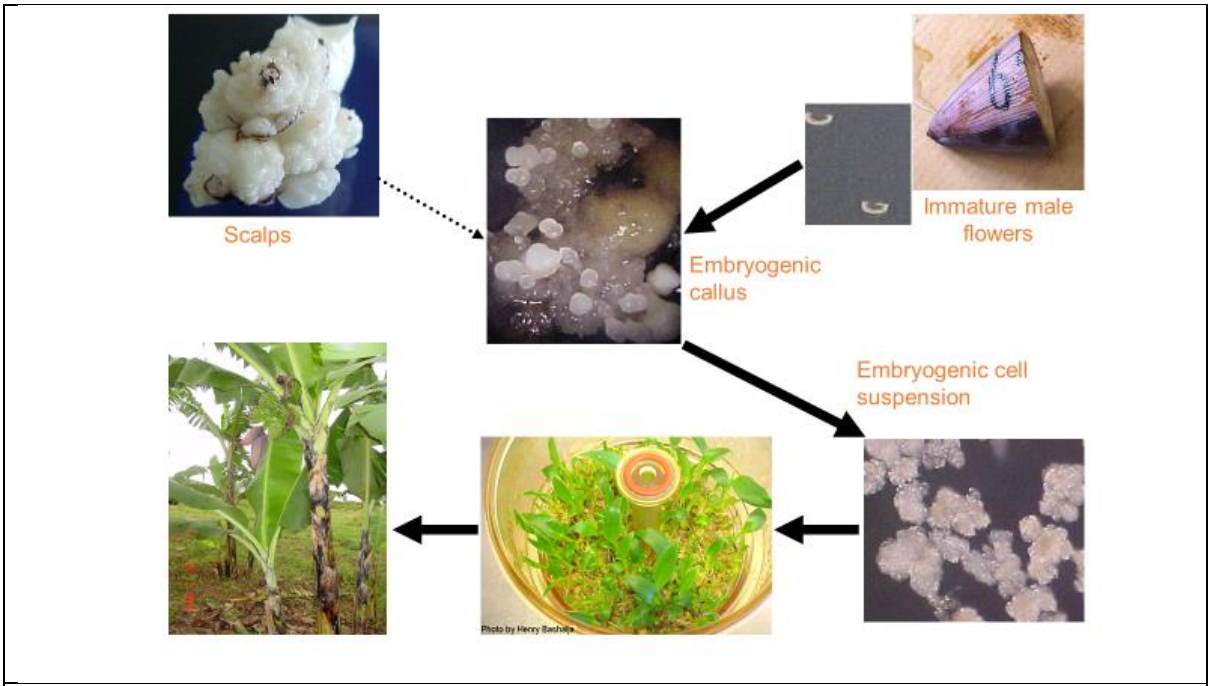


**Figure 4:1.** Development and regeneration of cell suspensions in banana

## 3.2 Agrobacterium- mediated transformation of ECSs of the banana

Upon developing embryogenic cell suspensions (ECSs), Sukali Ndizi ECS cell lines were transformed using the Centrifugation Assisted *Agrobacterium*-mediated Transformation system (CAAT) as previously reported by Khanna *et al*. (2004). Prior to transformation, settled cells in liquid MA2 were cultured for five days during which time *Agrobacterium* culture with *GUS* gene construct was cultured in LB broth supplemented with antibiotics. The five day-old settled cell volume of banana ECS was re-suspended in 10 ml activated *Agrobacterium* suspension, adjusted to 0.6 (OD600nm) and the mixture was co-cultured in the dark at 22°C for 5 days. Infected ECS were then washed in liquid MA2 selection media before transfer onto selective embryo formation (MA3) media.

Histochemical Gus assay for transient expression of the *gusA* reporter gene was performed five days after co-cultivation of ECS with the *A. tumefaciens* harbouring pGreen binary vector with *gusA* gene encoding the β- glucuronidase (GUS) enzyme in the transformed ECS of the banana cv. Sukali Ndizi.

Selection and regeneration of transgenic banana plants underwent through a series of subcultures whereby infected ECS were washed with liquid MA2 selection medium which were subsequently transferred and maintained on selective semi-solid MA3 medium until embryos development. The embryos were maintained on RD1 for prior to transfer of single embryos onto selective semi-solid MA4 media. Regenerated shoots were transferred onto anti-biotic free semi-solid proliferation media for shoot multiplication. Leaf tissue from putatively transgenic lines was collected from which genomic DNA was extracted and transgene integration confirmed endpoint PCR. In the case of *gusA*, histochemical GUS staining was also performed on leaves, stem and roots of greenhouse transgenic plants two months after weaning was also performed for detection of stable *gus* expression in mature tissues. Rooted lines of approximately 5 cm in height were weaned and placed in a high humidity chamber at 27°C in the biosafety level II green house for one month. The success of the transformation of Sukali Ndizi ECS was confirmed using histochemical GUS assay coupled with stereo microscopy. PCR confirmed plantlets were bulked and transferred to BSLII greenhouse for weaning and potting. Putatively transgenic GUS staining leaves were also positively assayed. The transformation protocol is summarised in Figure 4:2.

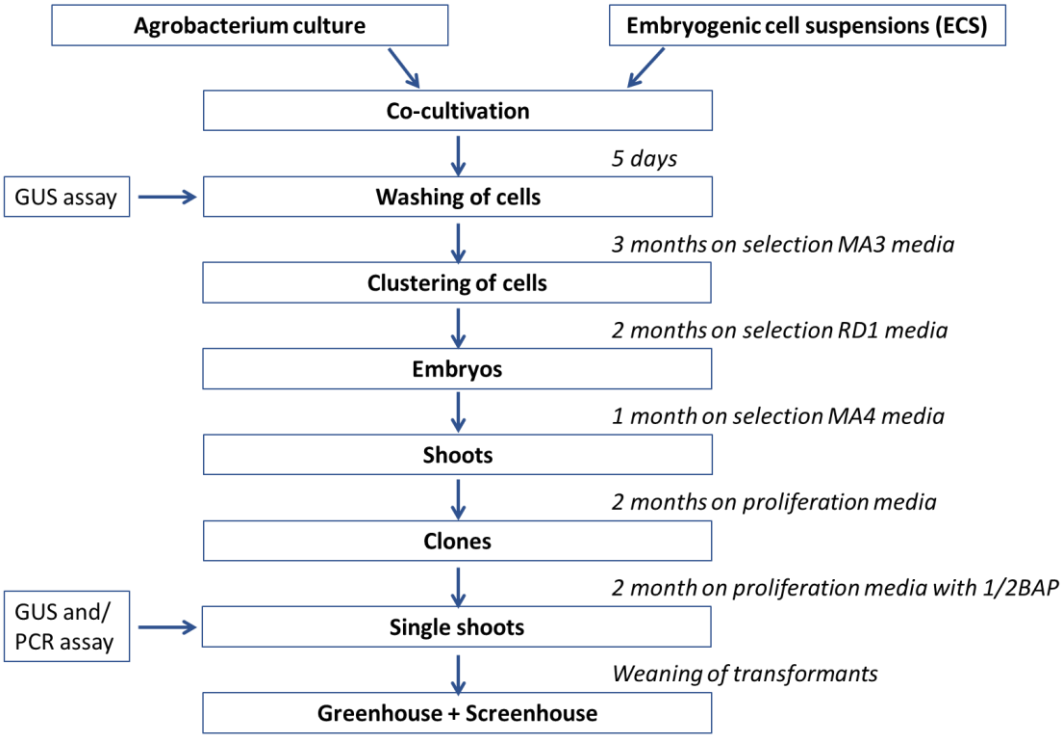


**Figure 4:2.** Schematic representation of the transformation system for banana

# 4 FROM TECHNOLOGY TRANSFER TO TECHNOLOGY DEVELOPMENT

At the inception of the Project, NARO's focus was to build capacity and partnerships and establish cell suspensions and genetic transformation systems for EAHBs for the improvement of banana. Through collaborations with partners, important genes were used to generate transgenic bananas which were characterised using morphological and molecular tools. Initially the Project focussed on addressing major constraints (including black Sigatoka, nematodes and weevils) for production of banana in Uganda.

## 4.1 Technology (Genes) transfer

As part of project implementation, NARO accessed papaya Cystatin (pCYST) genes from University of Pretoria, South Africa to transform target varieties for weevil resistance. NARO also accessed *Bacillus thuriengiensis* (Bt) genes (*Cry6A*) through collaboration with University of California (USA) where new Bt toxins had demonstrated nematocidal properties with a potential to increase nematode resistance in transgenic plants (Marroquin et al., 2000). Cystatin proteinase are expressed in plants in response to wounding and insect herbivore and form part of the native host-defense system. Proteinases are enzymes secreted into the gut of parasitic nematodes for breakdown of dietary proteins and proteinase inhibitors (Cystatin) restrain protein digestion by the nematodes (Urwin et al., 1997) and weevils (Kiggundu, 2008). Inability to digest dietary protein in the gut leads to protein deficiency and diminishes pest development, reproduction and survival. Toxicity to Bt protein arises when the molecule is cleaved by the insect's digestive proteases. The activated toxin attaches to the insect's mid gut epithelial cells where it creates pores in the cell membranes leading to gut leakage (Dorsch et al. 2002; Bravo et al., 2007).

Eventually, more genes were accessed under various projects that appreciated the capacity built by the NARO-Bioversity biotech project. For example, NARO accessed pro-vitamin A (PVA) enhancing genes from Queensland University of Technology (QUT) -Australia. In collaboration with the International Institute of Tropical Agriculture (IITA), NARO also acquired bacterial wilt resistance *hrap* and *pflp* genes from Academia Sinica-Taiwan through the African Agricultural Technology Foundation

(AATF). Other technologies like use of RNAi genes were obtained from Veganza for transgenic resistance to Fusarium wilt and black Sigatoka.

## 4.2 Technology (Genes) development

The NARO banana biotech team believes that there is a need to develop its own technologies to form a strong base for development of GMO products that meet the expectations of the farming communities. Not all technologies required will or can be accessed. For example, the RNAi technology did not confer Fusarium wilt transgenic resistance as expected. Yet the EAHBs in Uganda are immune to Foc1. Similarly, the black Sigatoka resistance genes engineered in Gros Mitchel at KUL-Belgium, readily succumbed to the disease in a CFT in Uganda. Also, if we successfully develop a transgenic Nakitembe with resistance to nematodes or weevils but is very susceptible to black Sigatoka, the product will perform very badly in black Sigatoka prone areas.

### 4.2.1 Cloning of *Cry6A* and C*pCYST* genes in PC1305.1

Based on the preliminary data, *Cry6A* and *CpCYST* both caused mortality of 72% and 63% respectively to 10-day old larvae fed on artificial diet. However, when these two proteins were combined, the mortality was 78%. Consequently, we developed a vector which combines the two genes because the two genes with different modes of action would give durable resistance in the transgenic plants.

The *Cry6A* and papaya cystatin genes were cloned as follows; using *SalI* and *BamHI,* restriction enzymes, *Cry6A* was excised from pBIN-JIT binary vector. Restriction sites for *Nco1* and *Pml1* were added to the sequence using PCR with developed primers. These sites enabled cloning of cry6A into pC1305.1, replacing gusplus gene using *NcoI* and *PmlI*. A colony PCR on pC1305.1 cry6A transformants was done to prove the presence of a construct. Papaya cystatin (*CysMut89*) gene under the 2x CaMV 35S and CaMV 35S terminator was obtained from pC1300 usingX*xbaI* and *KpnI*. It was then sub-cloned into multiple cloning sites of pCAMBIA1305.1cry6A. The resultant double construct was confirmed by sequencing both sides of the multiple cloning sites (Figure 6:1), confirming that both genes had been integrated.

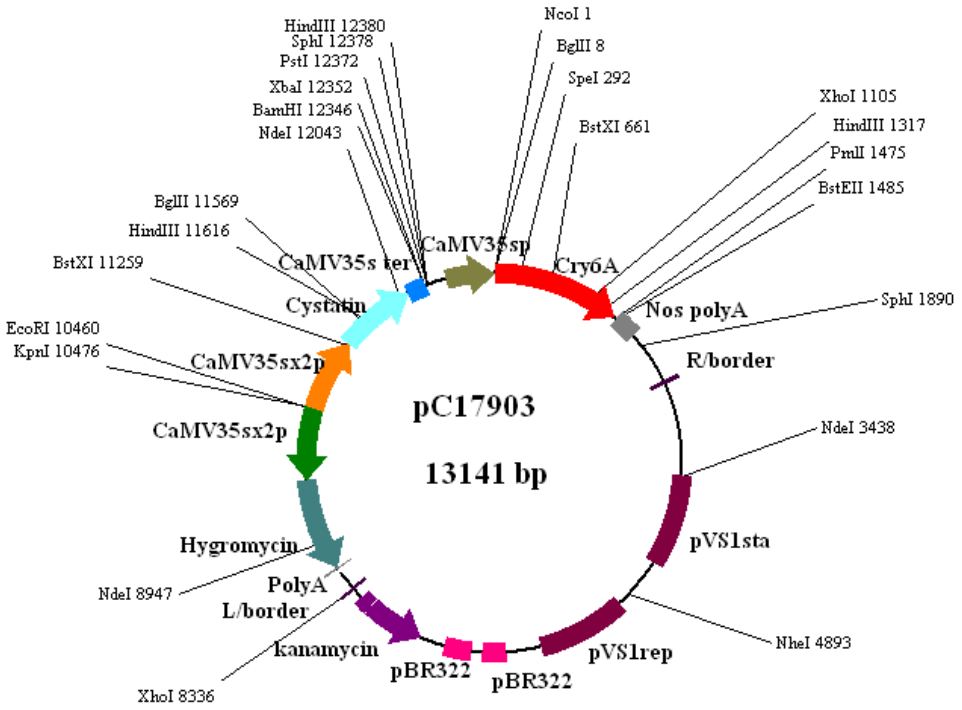


**Figure 5:1.** Final restriction map of the double construct with *BtCry6A* and *CpCyst*

### 4.2.2 Isolation of Foc resistance genes from Mbwazirume (AAA-EA)

Fusarium wilt (Panama disease), caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is responsible for significant losses of bananas worldwide. It decimated a whole Gros Michel dessert banana industry in Central and South America in the early 1850s. The Gros Michel was eventually replaced with Cavendish, resistant to Foc race1 and is now the dominant dessert banana on the world market. The Cavendish, itself, is susceptible to Foc TR4. Numerous control strategies have been devised to prevent damage caused by Fusarium wilt of bananas but have largely been unsuccessful in controlling the disease effectively (Herbert and Marx, 1990; Moore *et al*., 1995). Resistant bananas can be developed by conventional plant breeding or genetic modification (Ortiz and Swennen, 2014). Resistance to Foc is induced in the roots, which is the primary point of infection, to impede the progress of the fungus by means of cell wall strengthening and occlusion of the vascular vessels.

Next-generation RNA sequencing (RNA-Seq) was used to analyse the transcriptional changes taking place in banana roots following infection by Foc. RNA was extracted from the roots of the Foc race 1-susceptible banana ‘Sukali Ndizi’ (AAB), and the immune genotype ‘Mbwazirume’ (AAA), at four-time points (0, 48, 96 and 192 hrs)

post inoculation. Complementary DNA (cDNA) libraries derived from Foc race 1-infected roots were sequenced. The sequences were mapped onto the Musa reference genome, and 10136 genes were found to be differentially expressed (DEGs). Of these, 5640 genes were uniquely up-regulated, while 4496 genes were uniquely down-regulated in the three genotypes. DEGs were annotated with Gene Ontology terms and by pathway enrichment analysis.

The significant pathway categories identified included the following: ‘Metabolic’, ‘Ribosome’, ‘Plant–pathogen interaction’ and ‘Plant hormone signal transduction’ pathways. Several candidate genes and pathways that may contribute to Fusarium wilt resistance in banana were identified. Salicylic acid (phenolic compound that plays important role in immune response) and ethylene (plant immune response) - were stimulated in the ‘Plant hormone signal transduction’ pathways in all the three genotypes. Many genes were induced in response to Foc infection including 1) Oxidative bust, 2) lignifications i.e. cell wall strengthening, and 3) antifungal proteins. We are now focusing on antifungal genes that are differentially expressed by 'Mbwazirume' (resistant). There are some candidate genes including peroxidase 59 like gene, RGA3, Syntaxin 121 like, Endoglucanase1like, Bowman Birk type proteinase inhibitor and Chalcone synthase. RNA-seq analysis of three banana genotypes (‘Mbwazirume’, and ‘Sukali Ndizi’) to Foc race 1 showed that bananas respond to Foc race 1 infection by activating the ‘primary metabolic’ and ‘Ribosome’ pathways (Figure 6:2). The ‘primary Metabolic’ pathway enables the plant to meet the increased energy demands of the plant as it responds to pathogen attack, while the ‘Ribosome’ pathway provides for new ribosome's or changes in ribosome components to facilitate more rapid syntheses of defense proteins. This implies that nutrients are important factors in plant-disease interactions for the formation of mechanical barriers and synthesis of natural defence compounds (phytoalexins, antioxidants, and flavonoids).

The candidate genes are currently being cloned at NARL and the constructs will be evaluated for Foc transgenic resistance in Sukali Ndizi. Using the newly sequenced Musa genome, it is also possible to identify, isolate and use genes of interest including those that can address drought stress in banana. Once an array of genes is isolated by NARO technical teams, not only will researchers freely operate (Freedom to Operate, FTO) but the benefits of intellectual property rights (IPR) will attract more

collaborators who in turn provide additional technical and infrastructural development to NARO. Consumer support and public opinion towards GM foods would also be boosted because especially cisgene constructs are built by Ugandan researchers and transformed into crop cultivars of choice. The skills developed in these activities are adoptable by other NARO biotech initiatives. There are not many other donor agencies that would want to support gene discovery activities, yet that is where intellectual property benefits are. This necessitates continued support for NARO-Bioversity Biotechnology project as it serves greater national interest now and in future.

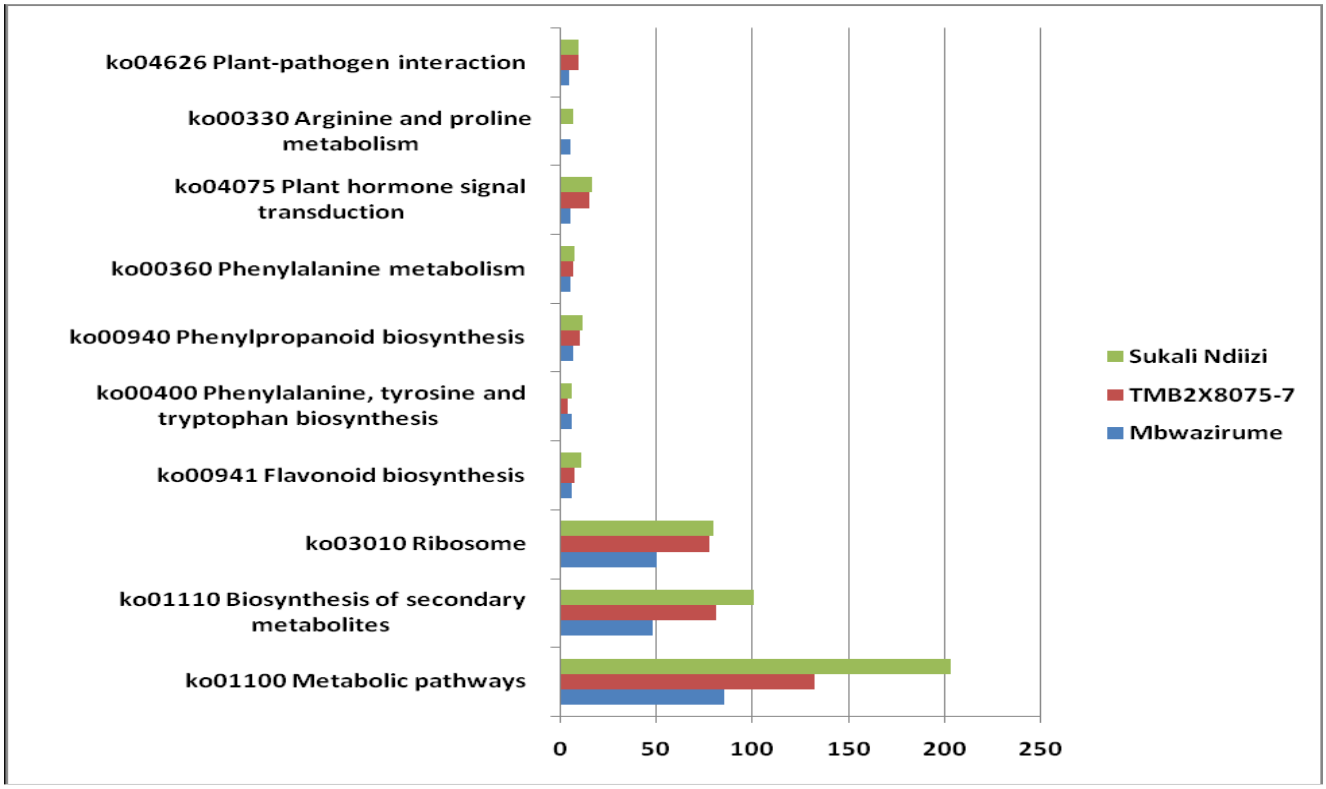


**Figure 5:2. Primary metabolic and ribosome pathways are differentially enriched in Mbwazirume, TMB2X8075-7 and Sukali Ndizi in response to Foc race 1 infection**

Source: Tendo's PhD thesis

# 5 PRODUCTS DEVELOPED UNDER THE PROJECT

## 5.1 Generation of cell suspensions

A regenerable cell suspension is the preferred starting materials for developing transgenic/cisgenic bananas. Cell suspensions of Sukali Ndizi, Gonja and Nakitembe were developed to facilitate genetic transformation of these cultivars with the papaya

cystatin and Bt (Cry6A) genes. The regenerable cell lines in the system (Figure 7:1) have been maintained through periodical subculture and 28 mL of packed cell volume of Nakitembe was used for transformation. Nakitembe lines 741, 743, 745 and 747 were cryopreserved and were confirmed to be retrievable. Basing on this competency, cell suspensions for NABIO hybrids are being developed.

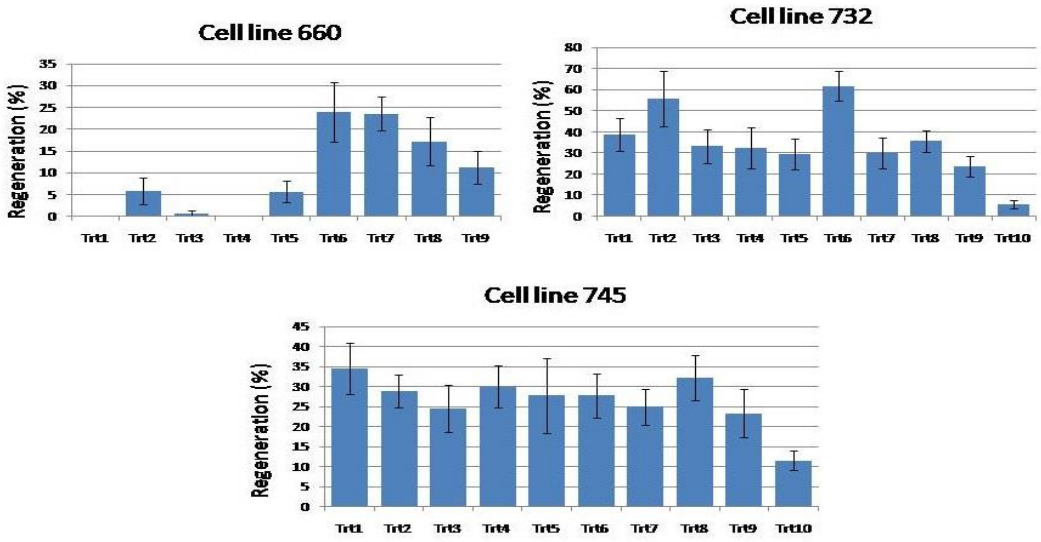


**Figure 6:1.** Regenerable Nakitembe cell lines

## 5.2 Gonja and Nakitembe with transgenic resistance to weevils and nematodes

Four products are expected under this activity namely; transgenic Gonja with weevil resistance, transgenic Gonja with nematode resistance, transgenic Nakitembe with weevil resistance and transgenic Nakitembe with nematode resistance. Two genes (*Cry6A* and *CpCYST*), singly and in combination, were used to confer resistance to weevils and nematodes in the two varieties - Gonja and Nakitembe. The genes have conferred more than 85% protection against weevil damage in more than 70 transgenic lines both varieties under screenhouse conditions and more than 90% protection to nematode infestation in 50 lines in Gonja and Nakitembe together. The protection levels for selection of resistant transgenic lines were set based on levels of nematode or weevil protection offered by the resistant checks of 'Yangambi' KM5 and Kayinja (Figure 7:2, 7:3 and 7:4). These lines will be advanced to confined field trials. The project gives hope to Uganda that the two major banana pests that have been responsible for destroying banana plantations in banana growing areas of Bunyoro, Buganda and Busoga will be effectively managed. Plantation longevity will be

increased and millions of farmers who have continuously planted bananas but harvested for only a short time will again sustainably have their stable plantations and bananas on their plate and market. If these products are supported through to release to farming communities, they would clear way for other NARO biotech products. The process will contribute to national regulatory framework support especially in product development to commercial release of GMO products. It could also contribute to IPR and Stewardship policies for Uganda, both very supportive to product development and release to end-users.

**Figure 6:2.** Transgenic lines with Cry6A and Cystatin genes showing resistance to weevils

**Figure 6:3.** Symptomatic diagram of criteria used to select transgenic lines with nematode resistance

## 5.3 Developing NABIO matooke hybrids

NABIO matooke hybrids were developed as part of this project. They are now being evaluated on-farm and selection is the final stage of the banana breeding process, leading to banana variety release. Four (4) of the NABIO hybrids: NABIO 0808 (M30), NABIO 0306 (M31), NABIO 0318 (M32) and NABIO 1011 (M33) selected from multi-location preliminary yield trials (Tumuhimbise *et al.* 2016), were multiplied and planted in replicated on farm trials in Jinja, Kamuli, Kabarole, Bushenyi and Mbarara districts. A similar trial to be used as check, weevil and nematode screening, as well as a source of male buds for the cell suspension development is at NARL-Kawanda. The hybrids are high yielding (30-40 tons/ha compared to 9 tons/ha that farmers get today); calculated income range of UGX 20-25 million /ha/year and other significant spill-over benefits for human health and positive environment impacts (Figure 7:5). As a key step towards variety release, one of the trials will be used for the distinctness, uniformity and stability (DUS) data collection by the MAAIF officials. These hybrids will be released into the banana farming communities of Uganda by end of 2018, making a key contribution to the strategic objectives of Uganda.

**Figure 6:4.** Three of the NABIO matooke hybrids due for release in 2018

## 5.4 Developing Fusarium wilt resistant apple banana (Ndizi) hybrids

Dessert bananas form the bulk of the bananas traded globally, valued at more than US$ 4 billion annually. However, they are less than 10% of the banana cultivar profile on-farm, majorly because of Fusarium wilt infestation. It is a disease that is very difficult to control except with resistant varieties. Introduced varieties of the FHIA series, have good resistance to Fusarium wilt, however, their consumer acceptability is poor. Two new apple banana hybrids (NAMU1 and NAMU2) and a local commercial cultivar susceptible to Foc race 1 but preferred by consumers (Sukali Ndizi) (Figure 7:6) were evaluated in replicated trials at the National Agricultural Research Laboratories, Kawanda in Uganda. Results showed that the bunch yields of the two new apple bananas were higher than those of check cultivars by over 50%. NAMU1 and Yangambi-KM5 showed no corm and pseudostem symptoms for Fusarium wilt. NAMU2, however, showed mild symptoms for the disease on corms while Sukali ndizi showed 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 Sukali Ndizi. The two hybrids together with others will be further evaluated on-farm and in the market before release. This will be a major milestone, in the development of dessert banana industry in Uganda.

NAMU1 NAMU2 Parent Sukali Ndizi

**Figure 6:5.** New apple banana hybrids resistant to Foc race 1

# 6 EXPLOITING THE SYNERGIES BETWEEN BIOTECHNOLOGICAL AND CONVENTIONAL BREEDING TOOLS

As the Project advanced, NARO Management advised the Project Steering Committee to link conventional breeding research tools with genetic engineering approaches in the quest to exploit synergies from the two approaches and hasten the research processes for producing new and resistant genotypes (Figure 7.1).

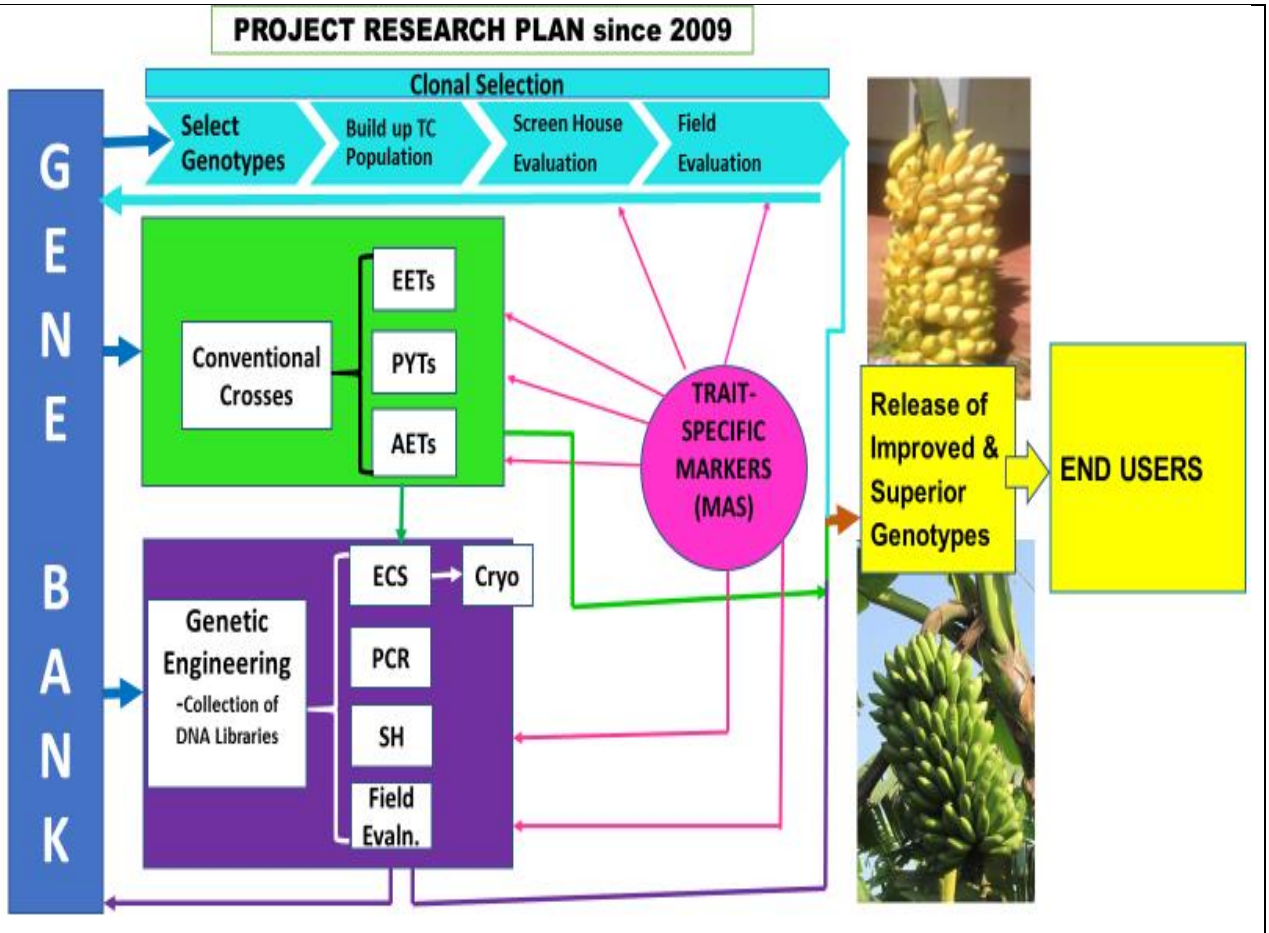


**Figure 7:1.** Integrating conventional and GE crop improvement tools

The strategy is aimed at enhancing the production and dissemination of improved hybrids resistant to the key biotic and abiotic stresses. All the three tools- clonal selection, conventional crosses and genetic engineering are now deployed by the project. Multi-trait resistance will be introgressed mainly through cross-breeding and clonal selection while single-trait resistance will be inserted into the hybrids through genetic engineering, all the selections mediated through markers.

## 6.1 Development of Markers

Beyond germplasm characterization, molecular genetics techniques have the potential to markedly enhance the efficiency of genetic improvement in Musa (Josh and Nayak, 2010). When molecular markers are co-inherited with physical traits, they are most likely associated with the genes underlying the trait. Nucleic acid sequence data obtained from expressed sequence tags (ESTs), resistance gene analogs (RGAs) and genome sequences can be used to develop genetic markers and maps, or to identify functional genes (Pillay et al., 2012). Markers and maps are useful for identifying and potentially cloning genes and quantitative trait loci (QTLs) of agricultural and biological significance.

### 6.1.1 Markers for Fusarium wilt resistance

Quantitative bulk segregant analysis (BSA) on the Diversity Arrays Technology Platform was used to identify markers associated with resistance genes. DNA pools of 40 resistant progenies and 40 susceptible progenies were used for BSA-DArT assay along with DNA from the resistant and the susceptible parents (Figure 8:2). A total of fourteen thousand three hundred and fifty four (14354) DArT markers were identified and sequenced from the resistant and susceptible bulks and the parents. One hundred and one DArT markers were in qualitative linkage disequilibrium, of which 13 markers were linked to resistance and 88 markers were associated with susceptibility to Fusarium wilt in Musa. These 101 distinct nucleotide sequences were mapped in silico, to either the annotated genes of the Musa genome (DH Pahang genes V1 database) or the full Musa reference genome sequence. Resistance gene (NBS–LRR type putative resistance protein CNL-B19-Orange block) occur within 20 kbps of a DArT marker (shown by the red arrow) associated with resistance to Foc race 1 in coupling on the Musa acuminata Pahang reference genome on Gbrowse (Figure 8:3). These candidate markers could be used in combination to predict the presence of resistance genes in banana breeding populations. Furthermore, these makers can also be used as indicators of genetic constitution in developing reliable banana improvement schemes.

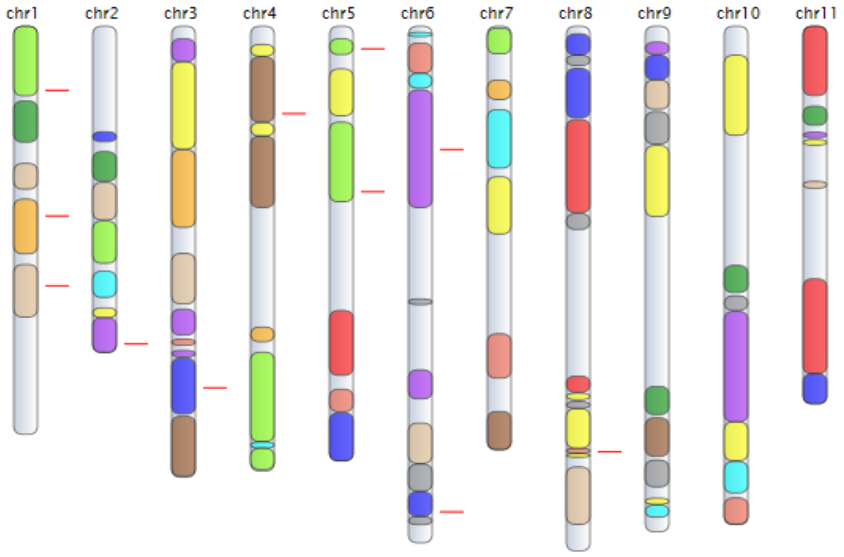


**Figure 7:2.** Location of the DArT markers linked to resistance to *Foc*, race 1 in coupling phase with significant similarity to the *Musa acuminata* Pahang reference genome

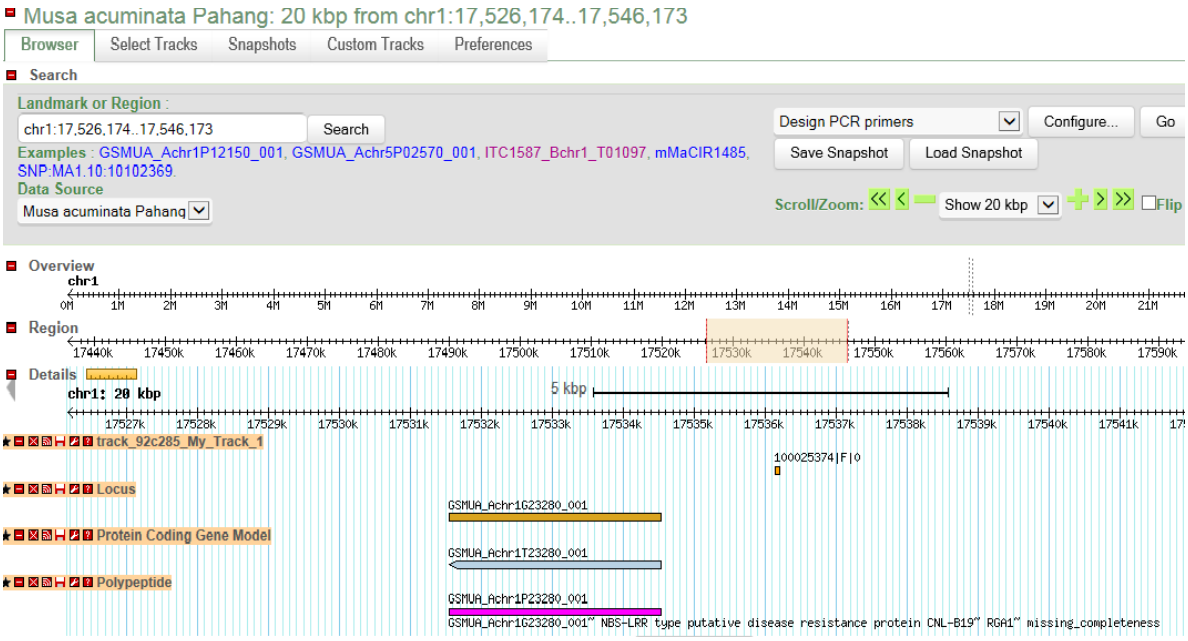


**Figure 7:3.** Resistance gene (NBS-LRR type putative resistance protein CNL-B19-Orange block) occur within 20kbps of a DarT marker

### 6.1.2 Markers for weevil and nematode resistance

Marker assisted selection (MAS) of genotypes of desired traits can save time in conventional breeding, especially for the banana with a long cropping cycle. This activity therefore mainly aimed at optimizing and validating the SSR markers using F2 segregating populations of crosses between a diploid weevil susceptible 'Kasaska'

and a resistant ‘Borneo’ (*Musa acuminata microcarpa*). Thirteen (13) primers were found to be polymorphic; four produced a single band and 9 two to three bands. Preliminary data collected from a screenhouse trial showed that most genotypes with low corm damage were also scored resistant with 3 primers (Table 7:1). The few genotypes that were scored as susceptible at molecular level still had low corm damage hence the need to collect more phenotypic data to obtain a clearer picture.

**Table 7.1.** Phenotypic and genotypic characterisation of banana lines for weevil resistance

| Genotype | Phenotype (% Corm damage) | Genotype | | | Remark |
|---|---|---|---|---|---|
| | | Marker F | Marker J | Marker Y | |
| 1 | 0.0 | RR | RR | RR | Resistant |
| 2 | 0.0 | RR | RR | RR | Resistant |
| 3 | 0.0 | Rr | RR | RR | Resistant |
| 4 | 0.5 | RR | RR | RR | Resistant |
| 6 | 0.0 | RR | RR | RR | Resistant |
| 7 | 2.6 | RR | RR | RR | Resistant |
| 9 | 3.8 | RR | RR | RR | Resistant |
| 10 | 0.6 | RR | RR | RR | Resistant |
| 11 | 0.0 | RR | RR | RR | Resistant |
| 12 | 1.3 | rr | rr | rr | Susceptible |
| 15 | 2.0 | Rr | rr | rr | Susceptible |
| 17 | 0.5 | RR | RR | RR | Resistant |
| 18 | 3.5 | Rr | RR | RR | Resistant |
| 20 | 4.9 | RR | RR | RR | Resistant |
| 22 | 1.7 | rr | rr | rr | Susceptible |
| 24 | 3.5 | RR | RR | RR | Resistant |
| 25 | 0.0 | Rr | RR | RR | Resistant |
| 27 | 0.6 | RR | RR | RR | Resistant |
| 30 | 3.9 | RR | RR | RR | Resistant |
| 31 | 5.0 | RR | RR | RR | Resistant |
| 33 | 15.1 | RR | RR | RR | Resistant |
| 50 | 0.9 | RR | RR | RR | Resistant |
| 53 | 0.5 | RR | RR | RR | Resistant |
| 54 | 0.5 | RR | RR | RR | Resistant |
| 59 | 9.1 | RR | RR | RR | Resistant |
| 60 | 2.1 | rr | rr | rr | Susceptible |

## 6.2 Integration of genetic engineering and conventional breeding tools

Overtime, the banana biotech team has also realised that single trait products can only serve the end-users for a short time or in localised geographical areas. After successful use of biotechnology tools to develop GMO bananas, the project now looks at development of multi-trait banana products, able to withstand various constraints. For example, bananas resistant to weevils and nematodes but susceptible to Banana Bacterial Wilt (BXW) will not stand for long if BXW is not controlled. There are two options for solving this challenge, which include stacking constructs of the promising technologies e.g. CRY6A/HRAP to attain weevil, nematode and BXW resistance, and the use of already improved banana hybrids from the conventional breeding programme where black sigatoka and weevil resistance are resolved and further fortify the hybrid with wilt resistance. In line with this strategy, NARO in collaboration with QUT already have an EAHB hybrid (M09), released for black sigatoka resistance and good consumer acceptability, transformed with PVA enhancing genes. The NARO-Bioversity project has now embarked on development of embryonic cell suspensions from NABIO hybrids. The NABIO hybrids are resistant to black Sigatoka and have high acceptance by consumers. When NABIO hybrids are transformed with a combined CRY6A/HRAP gene construct, we will have a banana product with resistance to BXW, nematodes, weevils and black Sigatoka and acceptable to consumers will be produced. With the supportive legislative environment, and now with the Biosafety and Biotechnology Law, it is envisaged to transform the best diploid (male parent) with BXW resistant genes. This will enable the development of all matooke hybrids with BXW resistance through a single crossing.

# 7 GERMPLASM; THE GENEPOOL FOR TODAY AND THE FUTURE

The main goal of *Musa* germplasm collections is to optimally harness the value of the collected materials for immediate and future use (Figure 8.1 and Table 8.1). Great attention was focussed on pre-breeding to enhance the use of germplasm collections. Pre-breeding contributes to the effective use of accessions and has been found necessary to produce materials that can easily be used by breeders. However, knowledge on the efficiency of the breeding system in bananas is also not complete, although essential for effective optimization of desired hybrid production. Under this project, resistance to weevils, nematodes, male fertility and pro-vitamin A content were considered very essential traits for improving the East African highland banana landraces (Table 8.2). Initially seven East African diploids were screened for weevil, nematode and black Sigatoka resistance. Feasibility of crossing the selected East African diploids with selected triploid landraces including assessing seed setting has been going on to generate a sufficient sample and steady number of seeds. From seed set results of 3 to 4 cycles, only two diploids produced most seeds with a wide range of female parents.

**Figure 8:1.** Musa Germplasm collection at MBAZARDI, Mbarara

**Table 8.1.** Ploidy analysis of the regional collection

| Ploidy level | Donations | ITC | Mission | Local collection | Unknown | Total |
|---|---|---|---|---|---|---|
| **Diploids** | 43 | 64 | 12 | 2 | 8 | 129 |
| **Triploids** | 66 | 50 | 8 | 22 | 5 | 151 |
| **Tetraploids** | 26 | 11 | 0 | 0 | 4 | 41 |
| **EAHB** | 46 | 25 | 0 | 111 | 1 | 183 |
| **Total** | 181 | 150 | 20 | 135 | 18 | 504 |

**Table 8.2.** Pro-vitamin A Content in diploids at the collection

| Sample ID | Sample name | BCE-FG | BCE-FR | HPLC RUN | Remark |
|---|---|---|---|---|---|
| MBA-001 | Musa balbisiana | 6.7 | 9.6 | Run50/51 | Analysed |
| MBA-002 | NABIO 318 | 9.3 | 11.4 | Run50/51 | Analysed |
| MBA-003 | NABIO306 | 8.1 | 12.3 | Run50/51 | Analysed |
| MBA-004 | TUUGIA 610 | 2.8 | 7.0 | Run50/51 | Analysed |
| MBA-005 | Pisang Jari Buaya | 27.0 | 31.0 | Run50/51 | Analysed |
| MBA-006 | NABIO 1011 | 8.8 | 12.9 | Run50/51 | Analysed |
| MBA-007 | Kamynyila | 2.6 | 3.4 | Run50/51 | Analysed |
| MBA-008 | NABIO1009 | 6.9 | 6.5 | Run50/51 | Analysed |
| MBA-009 | TMB2X 9722-1 | 32.2 | 39.5 | Run50/51 | Analysed |
| MBA-010 | Njuru | 4.0 | 5.4 | Run50/51 | Analysed |
| MBA-011 | Butuhan | 1.4 | 2.6 | Run50/51 | Analysed |
| MBA-012 | Khai Thong Ruang | 1.4 | 1.8 | Run50/51 | Analysed |
| MBA-013 | NABIO 1117 | 6.5 | 7.4 | Run51/51 | Analysed |

Activities of phenotyping banana germplasm for PVA and drought tolerance were initiated in October 2016. Results of PVA analysis for the 12 banana accessions out of 106 to be phenotyped have shown significant variability among accessions for PVA. Of the samples analysed so far, the highest PVA was recorded in a diploid TMB2X 9722-1 followed by Pisang Jari Buaya while the lowest PVA value was recorded in Khai Thong Ruang and Butuhan Intermediate (Table 8:1). Notable observations at this preliminary stage are; 1) NABIO hybrids have less than 10µg/g DW of beta carotene equivalent (BCE), at the level as that of Nakitembe currently being transformed by NARO and QUT collaboration. 2) There may be fertile diploids with high BCE (≥20µg/g DW) in the germplasm. There is therefore need to complete this analysis for the numerous diploids available in the germplasm.

For drought tolerance, initial efforts have been focused on searching for relevant literature. A summary of this literature corroborates what is already known that bananas are quite sensitive to drought; however, genotypes with the “B”genome are more tolerant to abiotic stresses than those solely based on the “A” genome. In particular, bananas with “ABB” genomes are more tolerant to drought and other abiotic stresses than other genotypes. Stomatal conductance, cell membrane stability, leaf emergence rate, rate of leaf senescence, and bunch yield under soil moisture deficit stress are some of the traits highlighted to be associated with drought tolerance. A PhD has been initiated to address this all-important stress.

# 8 EXTERNAL PROJECT REVIEW

The project Review was intended to assess the relevance, performance, management arrangements and success of the project. It looks at signs of potential impact of project activities on banana production and other beneficiaries and sustainability of results, including the contribution to capacity development (infrastructure and human). The review also identified lessons learned and was required to make recommendations that project partners and stakeholders might use to improve the design and implementation of subsequent project activities. The review observed and commended project management developing and implementing a framework that had a couple of strong pillars:

(a) **The integration of genetic engineering and conventional breeding tools**- can save time and maximize resource use. It also facilitates the building of a broad knowledge base about crop improvement since all scientists in the team are engaged in both approaches

(b) **The building of a collaborative structure that both south-south and south-north** which helped to broaden interactions and facilitate the acquisition and development of technologies while keeping participants abreast with new tools and approaches

(c) **The deliberate focus on the local East African Highland bananas for improvement** as well as a source of genes for crop improvement in the long-run is cheaper and promotes the relevance of technologies developed.

In terms of relevance and effectiveness of the project's strategy and approaches for the achievement of the project objectives, the Review Report noted that "*the project has successfully achieved set objectives and the implementation process towards realizing objectives was intertwined with activities that resulted into capacity building, technological advances and infra-structural development. Vertically within the project, staff have been trained on short courses to improve technology use and adoption (Table 4.2 of the review report) MSc and PhD to ensure capacity for science-led leadership and strategic planning (Table 4.3). The infrastructure development for genetic engineering, screen-house evaluations and tissue culture was developed. Horizontally, technicians have been trained from other commodity programs of NARO in aspects of embryonic cell suspensions, PCR analysis of transgenes and screen-house challenges of the transgenes. The Capacity development in response to*

*achievement of set project objectives is evident in the knowledge generated in terms of publications (Table 4.4) in peer-reviewed journals and other scholarly publications*"

In terms of its relevance to the national strategies, and relevance to direct beneficiaries, the Review Report, observed that the project aligned the research activities with Uganda priorities and planned activities to deliver public goods- knowledge, technologies, tools, methods, evidence, processes and platforms. The Uganda national priorities are clearly defined in the National Development Plan (NDP II) and Agriculture Sector Strategic Plan **(**ASSP 2015/16 – 2019/20). The latter specifically underlines- increased productivity; game-changing traits; climate smart agricultural technologies; value addition and agro-processing and discovery and application of new tools and methods.

On the direct beneficiaries of the project outputs to the people of Uganda, the report cited human capacity building (MSc, PhD, short courses) and infrastructure development at NARL, in support of project activities. Other NARO programs on sweet potatoes, cassava, potato and maize have benefited from trainings at NARL biotech laboratory. Furthermore, the project has developed and nurtured technical linkages with CG centres (IITA, CIAT), advanced research institutes (ARI) in UK, USA, Australia and the rest of Africa, resulting into access and exchange of knowledge and technologies.

The project also commented on the issue of cost-efficiency of project interventions and concluded that "*without the project, the advances made in tissue and cell culture, genetic engineering and conventional breeding would be nothing but a dream*!" Uganda now has all that it takes to develop new varieties in response to environment stresses, not just on banana but also on cassava, potato, sweet potato and maize. In addition, the project collaboration network which is so vital for technology advancement is now in place so that Uganda is at the fore front and a leader in banana R&D.

# 10. WORKSHOP REPORT

## Lessons learned

a. **Maintaining linkages between project objectives and national strategic objectives and priorities** to ensure project outputs contribute to national priorities. The project products thus far in the process of being released are both aimed at strengthening the commercialization of the banana farming systems- NAMU 1 and NAMU 2 as Foc Race 1 resistant varieties will greatly boost the dessert banana industry for the region. Similarly, the NABIO green cooking banana hybrids with resistance to black sigatoka, weevils and nematodes will improve the productivity of green-cooking banana systems.

b. **Awareness-raising within and outside partner organizations**. Most of the opposition to genetic engineering research is due to the lack of information and/or deliberate misinformation which capitalizes on the lack of information on GE. There is therefore a need for deliberate sharing out of correct information, packaged according to stakeholder categories, especially the media, the decision makers and the grass-root target beneficiaries.

c. **That for sustainability, research on national priorities such as food security, health and poverty alleviation** need to be developed as programs (and not projects) and for the medium - long term and funded primarily by national governments concerned. Other funders/donors can come on board later.

d. **That integrating genetic engineering and cross-breeding tools** can save time and maximize resource use. It also facilitates the building of a broad knowledge base about crop improvement since all scientists in the team are engaged in both approaches.

# 11. CONCLUSIONS AND RECOMMENDATIONS

**a. Human capacity building for biotechnology advancement:** the team now has expertise in banana embryonic cell suspensions (first successfully done at NARO-Uganda); constructs achieved and engineered them into target plants; and, the

analysis of the cisgenes/transgenes to confirm presence of the target genes in the transformed materials; bioinformatics and gene cloning skills, all essential for supporting and advancing biotechnological approaches. Only NARO-Uganda has this level of capacity in Africa as far as these crops are concerned.

**b. Leveraging other organizations to collaborate in the project:** As indicted in Table 4.1, the project brought in players from a number of regional and international institutions- Centre De Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), France; Katholieke Universiteit Leuven (KU-Leuven), Belgium; the John Innes Centre at the University of Leeds in UK; the International Institute of Tropical Agriculture (IITA), Nigeria; the Forestry and Agricultural Biotechnology Institute, University of Pretoria (FABI), South Africa; Queensland University of Technology (QUT) of Australia, University of California, San Diego (USA) and University of Georgia, Athens (USA) as well as other development partners: the Rockefeller Foundation, USAID and the Belgium Government. These new players brought in extra resources (human and financial) and technological know-how, which enable NARO-Uganda to advance rapidly on the agreed research agenda.

**c. Infrastructure development**: the main tissue culture laboratory was refurbished and equipped and a modern molecular biology laboratory was built and equipped for molecular research and practical application of genetic transformation. The laboratories are also supported by a back-up power generator to ensure that the laboratories remain functional in the face of possible power cuts on the national grid. The project focused on development and maintenance of infrastructure in the quest to provide a solid basis for new scientific advances.

**d. From technology transfer to technology development**: The project has now moved away from technology access to technology development to solve productivity constraints. The EAHBs are immune to (*Fusarium oxysporum cubensis*, race 1) (Foc1); the project in the last 3 years has been conducting activities aimed at isolating genes from Mbwazirume (EAHB landrace) that can be over expressed to confer resistance to the pathogen in susceptible cultivars. In this way, a number of genes were identified including the antifungal proteins (genes) that are differentially expressed by the resistant 'Mbwazirume'. A number of these genes are currently being

cloned at NARL and the constructs will be evaluated for Foc cisgenic resistance in the commercially important varieties such as Sukali-Ndizi, which succumb to Fusarium wilt.

**e. Management of Banana *Xanthomonas* Wilt (BXW) in east and central Africa**: In response to the national-level threat to the banana industry, the project joined in the search and development of effective interventions. NARO-Bioversity teams with additional McKnight Foundation funding developed and deployed a tool- Learning and Experimentation Approaches for Farmers (LEAFF), resulting into effective control of the disease. The successful control of BXW was the substance of the Farmers' Day celebrations on November 11, 2016, presided over by H.E. the President of Uganda.

**f. New varieties:** On the recommendation of the project Steering Committee to link/integrate conventional breeding research tools with genetic engineering approaches in the quest to exploit synergies from the two sets of tools/approaches and hasten the research processes for producing new and resistant genotypes (NARO-Bioversity hybrids - NABIOs) which are now on On-farm Evaluation stage in Kamuli, Kabarole, Bushenyi and Mbarara districts. The NABIOS are resistant to black sigatoka, weevils and nematodes. Concurrently, research is now in advanced stages to develop embryonic cell suspension from hybrids resistance to key biotic constraints in the process of engineering BXW-resistance genes into the hybrids.

## 12. FINANCIAL REPORT

Bioversity official financial reports are duly certified and signed by the responsible personnel. A duly certified Final Financial Report is submitted under separate cover.

## 13. CONCLUSION

Human resource skills and infrastructure for initial steps in the genetic engineering process have been developed by the project. Cell/tissue culture, transformation and greenhouse/field evaluations are well established and are now routinely executed. The

project team has now embarked on product development and gene isolation/cloning steps. With appropriate partnerships and sufficient support, the team hopes to accomplish the two steps in the next five years. The team will have a NARO genetically engineered banana and NARO owned genes. It is envisaged that the commercialization and stewardship step of the process will start after 2022 when the first genetically engineered banana will be released.

The project team is also involved in stacking promising technologies and transforming improved hybrids to develop products with desirable multiple traits to fit the banana producing and trading community needs. All these still require new skills in gene cloning and bioinformatics that NARO still need to support again for greater benefit of NARO's biotech activities. Uganda's national biotechnology strategy to ensure that the people of Uganda reap the full benefits of the revolution in biotechnology should be more valid today than 15 years ago. NARO's support to banana biotech work is already bearing fruits in terms skills development and products. With some more support, the project will be more able to service other NARO biotech initiatives and contribute the national strategy for adopting biotechnology to be fully achieved.

## 14. ACKNOWLEDGEMENTS

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List of publications (peer reviewed articles, proceedings, flyers, brochures etc.)

| No | Publications |
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| **2016** | |
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