**BIOSAFETY FORUM 2016**

***"Building Trust in the Regulatory System for Biotechnology"***


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**PROTEA HOTEL, KAMPALA**

# Forum Proceedings

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## LIST OF ACRONYMS

| | |
|---|---|
| AATF | African Agricultural Technology Foundation |
| ABNE | African Biosafety Network of Experts |
| ABSP-II | Agricultural Biotechnology Support Project II |
| ACMV | African Cassava Mosaic Virus |
| Bt | *Bacillus thuringiensis* |
| BXW | Banana Xanthomonas Wilt |
| CBSD | Cassava Brown Streak Disease |
| CFT | Confined Field Trial |
| CMD | Cassava Mosaic Disease |
| CMGs | Cassava Mosaic Gemini viruses |
| DNA | Deoxyribonucleic Acid |
| dsRNA | Double Stranded Ribonucleic Acid |
| EACMV | East African Cassava Mosaic Virus |
| GM / GMO | Genetically Modified / Genetically Modified Organism |
| HT | Herbicide Tolerance |
| IBC | Institutional Biosafety Committee |
| IITA | International Institute for Tropical Agriculture |
| MAAIF | Ministry of Agriculture, Animal Industry and Fisheries |
| MUWRP | Makerere University Walter Reed Project |
| NaCRRI | National Crops Resources Research Institute - Namulonge |
| NARL | National Agricultural Research Laboratories - Kawanda |
| NARO | National Agricultural Research Organization |
| NaSARRI | National Semi-Arid Agricultural Resources Research Institute - Serere |
| NBC | National Biosafety Committee |
| NDA | National Drugs Authority |
| PBS | Program for Biosafety Systems |
| RNA / RNAi | Ribonucleic Acid / Ribonucleic Acid Interference |
| SCIFODE | Science Foundation for Livelihoods and Development |
| siRNA | Small Interfering Ribonucleic Acid |
| UBIC | Uganda Biosciences Information Center |
| UNCST | Uganda National Council for Science and Technology |
| VAD / PVA | Vitamin A Deficiency / Pro-Vitamin A |

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**Executive Summary**

The Uganda National Council for Science and Technology (UNCST) organised the Biosafety Forum 2016 at Protea Hotel, Kampala (February 01 – 02, 2016). The theme of the forum was "Building Trust in the Regulatory System for Biotechnology". Over 70 participants who included the National Biosafety Committee (NBC) members, Institutional Biosafety Committee members, scientists/researchers, regulatory authorities, UNCST staff members, representatives from government agencies and the media attended the Forum.

Scientists discussed the progress made and the results of their genetic modification (GM) experiments. They illustrated the approaches undertaken while conducting research and gave future projections of their research and implications for biosafety regulation in Uganda.

The UNCST Executive Secretary, Dr. Peter Ndemere, informed participants that the Forum is part of UNCST's efforts to promote transparency on biosafety aspects of the GM research in the country for public accountability. The NBC Chairman, Prof. John Opuda-Asibo, noted the rapid advances in biotechnology to improve health, food security, and environmental sustainability and called for the strengthening of the biosafety regime for GM regulation. The UNCST Chairperson Dr. Theresa Sengooba agreed that biosafety is very important in assuring the public of the safety of GM products, about to come to market.

Prof. Charles Kwesiga, the Executive Director of the Uganda Industrial Research Institute, in his closing remarks, described the Forum as a timely event necessary to discuss opportunities for adoption of emerging scientific technologies aimed at developing the country. He commended UNCST for holding the Forum and thanked the scientists for the great work they are doing. Prof. Kwesiga expressed the need for more funding of promising GM research work.

Some of the key conclusions from the Forum were: the need for social economic data to guide decisions on commercialization of some of the promising GM products; the need for Uganda and UNCST in particular to ensure its biosafety regime keeps pace with the new advances in gene technology; and the need to ensure effective communication on issues pertaining to the GM technology.

The Forum was supported by the Program for Biosafety Systems (PBS) and Uganda Biosciences Information Center (UBIC).

# Part I—BACKGROUND TO THE FORUM

## 1.1 Introduction

As a National Competent Authority for biosafety in Uganda, the Uganda National Council for Science and Technology oversees and grants authorization for research and product development work involving genetically modified organisms. So far, 15 confined field experiments of genetically modified crop trials have been approved by the National Biosafety Committee at UNCST since 2005. These crops include cotton, maize, cassava, banana, sweet potato, potato, and rice. The experiments are conducted within the National Agricultural Research Organization's institutes, which are also the primary applicants. The sites for the experiments are in the districts of Serere, Kasese, Kabale, and Wakiso (Kawanda and Namulonge). These experiments are conducted in collaboration with a number of regional and international partners.

Although several experiments with GM plants have been approved and are ongoing and the respective scientists report (on an individual project basis) to the regulatory authority, there had been no forum where the researchers involved share their scientific progress and the results of the experiments with peers and regulatory authorities. While researchers/scientists submit progress reports of their work to the NBC and UNCST, this is not sufficient to reflect on some of the issues such as the scientific validity of the approaches taken, the results obtained, and implications for biosafety. The Biosafety Forum was thus proposed as an annual platform for the NBC, IBCs, UNCST, and researchers/scientists to converge and discuss the scientific merit of their work, and reflect on the biosafety regulation in the country.

The forum was also intended to keep the regulatory authorities abreast with new and emerging techniques in GM technology, and discuss the effects these may have on the biosafety regulatory process. Therefore, the Biosafety Forum was not only to provide constructive criticism and feedback on the agricultural biotechnology research and development work that is ongoing, but also to increase the participants' knowledge and understanding of the global scientific trends of GM technology and the challenges it presents for biosafety regulation.

## 1.2 Objectives

The objectives for the Forum 2016 were:

a. To share the scientific progress and the results of GM experiments with peers and regulatory authorities.
b. To enhance the interaction between the NBC, IBCs, national regulatory agencies, key decision makers in government, and biotechnology scientists/researchers in Uganda; and
c. To identify priorities for biosafety research, policy and regulatory development in Uganda.

## 1.3 Expectations

The expectations for the Biosafety Forum 2016 were:

a) UNCST, NBC and other regulatory agencies would be more knowledgeable about the current scientific developments in GM technology, and better equipped to make sound regulatory decisions thereof;
b) There would be improved implementation and compliance with national biosafety guidelines and regulations; and,
c) Proceedings with extended abstracts of the Forum presentations will be published.

# Part II—OPENING SESSION/REMARKS, CLOSING REMARKS

## 2.1 Opening Plenary

### 2.1.1 Welcome Remarks by the Executive Secretary, UNCST

Dr. Peter Ndemere, the Executive Secretary of Uganda National Council for Science and technology welcomed participants to the Biosafety Forum 2016. He observed that Uganda is one of the most active countries in Africa conducting field testing of GM crops namely; cotton, maize, cassava, banana, sweet potato, potato, and rice. Therefore, the UNCST mooted the idea of a Forum to serve a platform where scientists working with GMOs interact with their peers; and also with the biosafety regulatory authorities to promote openness in the biosafety regulatory system, and public accountability for the scientific research done. In conclusion, Dr. Ndemere reaffirmed UNCST's commitment to providing a supportive regulatory system aimed at promoting transparency of biosafety in the country for public accountability.

#### 2.1.2 Opening remarks by Chairperson, NBC, Prof. Opuda-Asibo

Prof. Opuda, welcomed participants to the Biosafety Forum and thanked them for taking time off their schedules to participate in the deliberations.. He observed that NBC resolved that it was imperative to hold a biosafety forum to build public trust in the biotechnology regulatory system of Uganda.

Giving the historical perspective, he noted that recombinant DNA technology started in 1976 in California and over the years, great strides have been made in this field over a period spanning three decades. He further observed that though progress has been made in this scientific field, there has been a growing antipathy towards some of the crops and crop products derived from them. He attributed the antipathy, low embracement and eventual minimal adoption of biotechnology in some regions of the world to the natural emotion of fear by humankind, ethical issues, religion, and perceived adverse consequences to biodiversity. He remarked that on a positive side, a lot of scientifically documented advancements in health, food security, industry and environment are being accomplished with utilization of products of modern biotechnology.

He noted that the NBC has authorized several CFTs of GM crops and added that NBC was prepared to ensure that the research is done in accordance to standard operating procedures and the national guidelines for field trials of genetically engineered plants.

In conclusion Prof. Opuda - Asibo noted that Uganda, being party to Cartagena protocol has an obligation to regulate biotechnology and also to harmonize its local existing policies and laws to suit international obligations. He concluded by requesting the government of Uganda

to pass the Biosafety and Biotechnology Bill, 2012 to provide a stable and long-lasting framework for biotechnology innovations and developments.

### 2.1.3 Opening remarks by Chairperson UNCST, Dr. Theresa Sengooba

The Chairperson Dr. Sengooba welcomed all participants to the biosafety forum 2016 and expressed delight and profound expectations for the outcomes of Biosafety forum 2016. She noted that biosafety regulations form an important and to advance modern biotechnology**.** She remarked that there is need to have a strong legal resource base and economic resource base in strengthening the biosafety regulation.

Dr. Sengooba acknowledged the role played by UNCST in ensuring biosafety and further mentioned that it had made a great contribution to human resource and capacity building to members the NBC, IBCs, and regulators. She added that UNCST has contributed to institutional infrastructural development despite of some bottlenecks like limited financial capacity and bans on biotechnology products. She noted that the approved CFTs were being conducted in accordance with International legal agreements like the Cartagena protocol.

In conclusion, Dr. Ssengoba called for cooperation and understanding amongst the scientists in order to reach an amenable scientific consensus to illustrate the much need for biotechnology for development before officially opening the forum.

## 2.2 Closing remarks

### 2.2.1 Remarks from the Chairperson of UNCST Dr. Theresa Sengooba

Closing remarks were made by the Chairperson of UNCST Dr. Theresa Sengooba, Executive Secretray of UNCST Dr. Peter Ndemere and Chief guest Dr. Charles Kwesiga. Dr Sengooba, thanked participants for sparing their valuable time to attend the forum and thanked the organizing team of the forum for the wonderful job done. She also commended scientists for the work being done and current efforts underway. She however observed that most of the work being done is funded by international donors and appealed to Prof. Kwesiga, who also serves as a Presidential advisor, to inform government to intervene and support the scientific research work. She called upon the government to urgently pass the Biosafety and Biotechnology Bill to enhance the regulatory framework of biotechnology and its further harmonization with International Obligations, particularly the Cartagena protocol. Finally, she called upon the scientists to adopt a simple communication strategy in order to easily allow the local and non-scientists to effectively get the message in the scientific work. Dr Ndemere, thanked the scientists, participants and all stakeholders for attending this forum and promised support for further engagements in biosafety. Prof Kwesiga described the Forum as a timely event necessary to discuss opportunities for adoption of emerging scientific technologies aimed at developing the country. He commended UNCST for holding the Forum and thanked the scientists for the great work they are doing. Prof. Kwesiga expressed the need for more funding of promising GM research work. He recommended the need for scientists and institutions to seek international collaborations and partnerships.

# Part III—OBSERVATIONS/MATTERS ARISING/ RECOMMENDATIONS

The forum participants noted the emerging quick pace of trends of technological developments in the aspect of biotechnology and there was a need for the country to mount an appropriate legal, financial, scientific and technological response. Participants noted the lack of legal framework for biosafety as the greatest stumbling obstacle to progress. They further noted that Ugandan lawyers lacked basic training in aspects of biosafety. In addition the population of Uganda at large lack information and knowledge in biosafety and biotechnology and existing communication strategies for biosafety are generally inadequate. In the area of research, it was noted that most researchers use imported transformed transgenic varieties of crops for experimentation. In addition researchers lacked a comparison and a comparative view on the economic trends of GM crops versus non GM crops in their research studies. Limited financial support by government to GM experimentation hinders progress and sustainability

1. A need for the government to pass the Biotechnology and Biosafety Bill, 2012 into law and to harmonize it with the Cartagena protocol.
2. A need for regulators in biotechnology to network and receive requisite trainings in new emerging information regarding technological advancements in biotechnology innovations.
3. A need to train legal practitioners the science of biotechnology since most legal experts have limited knowledge and expertise in biosafety and biotechnology in order to strengthen the legal flame work.
4. A need to educate and create also awareness campaigns on the science of biotechnology
5. A need to formulate a simple communication strategy while communicating the science of technology for easy understanding.
6. A need to use the local crop varieties in introgression and transformation events other than relying solely on importing transformed varieties in order to enhance sustainability.
7. A need to ensure compilation of the social –economic analysis of transgenic varieties versus local varieties since there will be commercialization after Biotechnology and Biosafety is enacted into law as stipulated in the Cartagena Protocol.
8. A need to undertake adequate research on non-target organisms on all GM studies to get a better assessment on risk assessment and risk management in the ecosystem,
9. A need to test efficacy of the single events before progressing to stacked genes to enhance the effectiveness of results' validity during experimentation.
10. A need to explain benefits and merits of GM technology over traditional farming to the public and most especially to farmers to fully understand the long term implications and benefits of adoption of the GM technology
11. A need to conduct multi-locational trials (for genetically modified maize and banana varieties) on specific studies and in different agro ecologies to fully understand the performance of the transformed varieties in other regions.
12. A need for researchers to get acquainted with the importance of intellectual property rights in the GM experimentation work in order to protect the innovations
13. A need for government support towards the GM technology research
14. Regulators and Scientists need to foster international partnerships with other countries in establishment of a functional regulatory framework and conducting GM work

15. Observation was made to notify NBC on the Medical biotechnology work being done in Uganda.

# Part IV- EXTENDED ABSTRACTS

## 4.1 Keynote address: Gene technology: Past, Present and Future

*Dr Andrew Kiggundu,*
*National Agricultural Research Laboratories - Kawanda.*

The present large number of crops currently grown and livestock reared were domesticated and adopted by our forefathers through conventional breeding. There are however some limitations of this process and this has led to the development of other better means of intervention, which include biotechnology.

Some of the new breeding techniques like double haploid technology has made it possible to breed rice, maize, cassava and banana faster and effectively. There are different processes that take place in the development of genetically modified plants in the laboratory. For example, animal cloning is being done worldwide and the latest example is the genetically engineered Goat Producing Atryn, GM mosquitoes *Aedes aegypti* are being designed to fight against malaria causing parasites.

Other emerging new developments in genetic engineering include: Gene silencing, Gene editing – CRISPR, Gene drives, Synthetic genomes – Synthetic life and DIY-Bio.

The application of biotechnology in the medical field has helped to solve problems that have been a very big problem such as the development of the HIV vaccine through gene-editing and the treatment of leukemia through gene editing.

In conclusion, the field of genomics is moving so rapidly and is helped by increasing power of information and communication technologies (ICT) that is also becoming cheaper as well as data available freely in online scientific repositories supported by both public and private investment in biosciences.

## 4.2 Current status of play of international biosafety laws and their implications for biotechnology development in Uganda

*Ms. Harriet Ityang*
*Ministry of Justice and Constitutional Affairs.*

There are nine international bodies that regulate and govern different aspects of food safety and agricultural biotechnology. Five of these bodies are science based organisations namely- The International Plant Protection Convention (IPPC) which deals with issues of Pests and pathogens in crops; The International Epizootics Organisation (OIE) which deals with Pests and Pathogens in animals; The Codex Alimentarius (Codex) which deals with Food Standards and Labels The Food and Agricultural Organisation (FAO) which is responsible for Food security programs; and The World Health Organisation (WHO) which is a Health Science Policy body.

Of the remaining four bodies, the World Trade Organisation (WTO) is a trade-based organisation which deals with Trade rules for goods and dispute settlement mechanism while the remaining three organisations have broader objectives such as environmental protection and other political or social goals and these include The Organisation of Economic Co-operation and Development (OECD) which harmonizes standards and policies; The Convention on Biological Diversity (CBD which handles biodiversity related issues and the Cartagena Biosafety Protocol (BSP) which deals with the transboundary movements of living modified organisms.

In conclusion, there is great need to have our own biosafety law in Uganda which is harmonised with other international laws. The absence of such policy framework on the role of the life sciences in achieving the global objectives of poverty reduction, health care, and environmental conservation is a serious hindrance in the quest Uganda to take advantage of available opportunities for its economic transformation.

## 4.3 Challenges of Biosafety Regulation in Africa: "NEPAD Agency/ABNE Experience"

*Mr. Sunday Igu Rock Akile & Dr. Silas Obukosia,*
*NEPAD Agency African Biosafety Network of Expertise (ABNE)*

**Introduction**

In the present world situation, the limits of human capability to produce sufficient food by conventional crop production, have been plagued with high input but low yield through diseases and pests infestations which has prompted the search for solutions globally. It is realized that biotechnology applications could lead to dramatic and rapid changes especially towards achieving human food problems once harnessed safely. Safe harnessing of Biotechnology applications calls for the establishment of regulatory systems and currently many African countries are grappling with the challenge of establishing functional biosafety regulatory systems.

Having considered the potential to contribute towards increased agricultural productivity and help mitigate food security and climate change challenges the continent faces, the African Ministerial Council on Science and Technology (AMCOST) in 2008 pronounced that modern biotech could be the a technology for Africa. Premised on that ground, the African Union-NEPAD Planning and Coordinating Agency (NPCA) started to place a high priority on the safe use and management of biotechnology. As a specialized centre of the AU-NPCA, African Biosafety Network of Expertise (ABNE) is mandated to support African countries in building functional biosafety systems that will ensure the responsible and safe development, adoption and utilization of agricultural biotechnology products.

A functional biosafety system of a country on the continent at minimum should entail policy provisions regarding biotechnology and its safe deployment, a legislative framework, the administrative establishment, the infrastructure in place, the capacity to review biosafety applications, the capacity to monitor and evaluate. As policy is key in setting out clearly the intentions and values of the government, the policy positions for enforceability will have to be reduced into legislations, regulations, ordinances and guidelines. A Biosafety Administrative establishment is key to successful handling of applications and making decisions over such applications. A good administration must have entail personal and equipment for keeping institutional memory. The system must not only put in place a capable and well resource evaluation and monitoring system but It should also put in place mechanisms of ensuring public participation.

**Challenges of Biosafety Regulation in Africa**

**1. The African Model law on Biosafety**

The idea arose among the African negotiators of the Cartagena Protocol on Biosafety to develop a biosafety model law to provide guidance for the development of domestic biosafety

laws. The first draft of this model law was developed by an OAU workshop of experts from Africa and other developing countries in Addis Ababa in June 1999. This draft was based on the proposal of the African Group for the biosafety protocol, which it submitted to the CBD secretariat in 1996. The provisions of the African model law had more concern for risk and safety measures than recognizing the benefits the technology could post to economies on the continent in terms of revenue and the solutions to the food insecurity. The African Model Law influenced policy and the legal drafting in many African countries such as Ethiopia, Ghana, Mali, Tanzania and many others. To date the positions of some these countries have presented a challenge in biosafety regulation especially with respect to policy formulation and legislation.

Policies normally informs legislation drafting and once the policy is not good, expect a law that is not good as well- For example; the policy of a particular country's position on GMOs determines the nature of the legislation such a country will have. Policies the scope of a regulatory authority, the role of stakeholders, the public and other government departments and agencies will all be reflective of the law, Most African countries aimed at risk and safety less of recognizing benefits that biotechnology could post to their national revenues or as a tool for food security. The legislative development path for most African countries did not enable the make the choice of utilizing or harnessing plant biotechnology safely. Most legislations provided punitive measures against omissions and errors (Imprisonment or fine or both). The text of some legal documents are ambiguous; leading to strict un-implementable national laws e.g Socio –economic, cultural, traditional considerations in risk assessment. With the foregoing picture of both policy and legislation, biosafety regulation is inevitably a challenge.

**2. Resource Limitation**

Human resource development in some countries has been at its lowest. Most Africa countries cannot review applications and their regulatory bodies are not properly constituted to have technical advisory committees. In addition, they have inadequate capacity for inspections and monitoring. Furthermore, resource allocation to the regulatory agencies is adhoc and inadequate and regulators are poorly remunerated so they often move on to greener pastures- the turn-out rate is high.

**3. Civil Activism**

1. Civil society groups in most countries have become - Anti-GM groups and keep shaping the debate as they advocate against GMOs (unfortunately some of the ground of their advocacy is un-scientific) like in Burkina Faso. Consequently, legal suits against the regulatory agencies has become a new trend of frustrating regulations (Civil Society group seeking injunctive relieves from courts of law as was been witnessed in Ghana and Kenya in 2015 where applications for injunctive relief were both dismissed in the countries for lack of merit. However, all in all, these cases cause setbacks towards an amicable biosafety regulation.

**4. Government Decisions**

There have been instances where radical decisions by Governments in some countries have given challenges to biosafety regulation process. For example the Executive decision by the Sudan government to develop and give a boost to her cotton Industry is a case in point. Although there was merit for the decision, there was however no much of public participation. Hence it raised the concerns over environmental democracy because a good biosafety regulatory system should give room for public participation. Also in Kenya whereas the National Biosafety Authority Board is mandated to review, make decisions on Biosafety

regulations, it was not given any chance before the ban was pronounced by cabinet on all GMO imports.

**1. Lack of infrastructure**

Many governments in Africa do not have laboratories to test for GM traits in crops. Such countries cannot stand the minimum standards that are set out in the Cartagena protocol and so cannot benefit from some biotechnology applications, which demand rigorous risk assessment review.

**2. Porous borders**

One of Africa's main challenges is having porous borders and this makes biosafety regulation a big challenge. It often leads to the abuse of the biosafety laws due weak monitoring, supervision and infrastructure. Poor border controls have made it possible for LMOs to move across international borders un noticed. Such cases have been noted between the countries hereunder where traces of GM material have been found other international borders like the

2. Swaziland and South Africa border, the Ethiopian border with Sudan (Gambela region) and the Ghana from Burkina Faso border.

**7. Regional groupings**

Regional groupings have tended to drive the agenda and interest of such grouping for example; The West African Monetary and Economic Union (*Union Economique et Monétaire de l'Afrique de l'Ouest*) - UEMOA = WAEMU are expressing the need to maintain a more Risk and Safety approach than Burkina Faso which had already commercialized GM Bt Cotton. This no doubt presents a challenge in biosafety regulation in the region . The Economic Community of West African States (Communauté Economique des Etats de l'Afrique de l'Ouest) ECOWAS position from the francophone speaking countries has complicated regulation of biosafety regionally as the Biosafety Secretariat at ECOWAS in Abuja as been rendered almost irrelevant thus creating varying positions and interests between the Francophone speaking and Anglophone speaking countries in West Africa..

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## 4.4 Understanding key points of contention within the proposed national biotechnology and biosafety


*Mr. Herbert Oloka,*
*Program for Biosafety Systems (PBS)*


The Program for Biosafety Systems (PBS) supports partner countries in Africa and Asia in the responsible development and use of biotechnology. Managed by the International Food Policy

Research Institute (IFPRI), PBS works with countries interested in using biotechnology to enhance agricultural innovation. PBS also, provides comprehensive, integrated approach to development of evidence-based biosafety regulatory frameworks works with the Government of Uganda in establishing policy, research review procedures, biosafety training, and regulatory framework development. Under PBS guidance, consultations about the biotechnology and biosafety bill by parliament were concluded. The target group included policymakers, scientists, academia, farmers, media, civil society, religious leaders, and other regulatory agencies.
Different key considerations from stakeholders, different changes proposed by stakeholders were received and considered. The main point of contention was that this law is not needed now or in the future, they believed the law would open Uganda to 'mass introduction of GM crops', which they are against. After discussion, it was agreed that main purpose of enacting this law is to regulate the use of modern biotechnology tools therefore it is important to have this law.

Some stakeholders argued that the definition provided for in the bill is not satisfactory, however discussions concluded that if it's necessary some clauses may be added to match the interests of many and also some argued that the title should be restricted to GMOs but this was refuted citing that it was designed in consideration with the current policy therefore this would imply that some biotechnology tools would be left out therefore it was preferred that the title would be maintained.

Other issues discussed in the consultation included: labeling, designation of the competent authority, responsible ministry, minimum period for decision making, appeals mechanism, expected review of applications, offences and penalties, liability and redress.

In the way forward it was suggested that many of the proposals made by stakeholders can be utilized to enrich the law. The bill is yet to be debated and passed by Parliament, so the opportunity exists for the Minister and MPs to amend the law as appropriate. Some of the proposals are likely to be approved by the Gov't when the bill is debated and finally consensus achieved among many policy makers on the key issues.

## 4.5 Communicating biotechnology more effectively

*Dr. Barbara M. Zawedde,*
*Uganda Biosciences Information Centre (UBIC)*

Uganda Biosciences Information Center (UBIC) is a NARO-led information hub established in 2013 with an aim to be a one-stop center for information sharing on agricultural research in Uganda. In relation to biotechnology, UBIC aims to facilitate informed decision-making by contributing to public understanding of agricultural biotechnology, and build public confidence in the regulatory system.

There is a need for biotechnology communication because there is persistent controversy about some of the biotechnology tools that has resulted in persistence doubt, mistrust and uncertainty. Therefore there is need to have an independent or credible body that will give accurate information about biotechnology.

There are five key components of effective communication including situation/stakeholders analysis, message packaging, the messengers, message delivery and message absorption. In the situation analysis it is important to understand the environmental status and to conduct a

thorough comprehensive stakeholders' net-mapping depending on level of influence and interest.

Packaging of the message should support keeping it simple and attractive in order to easily reach out to the Lehman. Use simple words such as better harvest instead of higher yields, better nutrition or quality instead of bio-fortified products among others.

There are a number of communication principles that must be followed during message packaging. The 3:3 principles involves having three parts of a message for example if developing a message on food biotechnology; you may include a consumer benefit, safety message and a sustainability message. For each message prepare three supporting facts or evidence. It is critical to take note and be careful about non-verbal messages such as using a photo of armed guards at a a confined field trial site. Such photos need explanatory captions to avoid mis-interpretation.

When choosing a messenger, it is critical to select someone who is knowledgeable and considered credible by the target audience. In Uganda it is evident that the public considered scientists to be a credible source of information about biotechnology. Therefore there is need to build a critical mass of scientists to act as spokespersons for the work they are conducting. It is also important to pair scientists with communication specialists for example mass media personnel so that they help to interpret and relay the science into a language that will be appreciated by the public. Influential champions from our target audiences are also credible messengers for delivering the message in a way that will be appreciated.

Messengers need to understand that trust is critical for absorption and appreciation of the message, and in order to build trust, the messenger needs to show empathy and care to their audience. According to research by psychologist Marshall Rosenberg, founder of Non-Violent Communication, if you show empathy conflict resolution is reached 50% faster.

Mass media especially radio is a critical media of message delivery for biotechnology communication. According to the survey carried out by UBIC, it was found out that all respondents use radio as a source of agriculture information.

Absorption of the message is improved when there is high trust, when people feel in control of situations, when they can relate with or see benefits to them, and when the whole situation is seen to be fair and equitable. Simple messages, repeated often by a variety of trusted voices are more likely to be accepted (Murray, 2013).

In conclusion, scientists and relevant Government agencies need to play their role in biotechnology communication. The media should to continue interacting with the scientists so that they help stakeholders to access accurate information from credible sources before they make decisions.

## 4.6 The performance of Bt maize event MON810 against Stemborer pests under Ugandan conditions

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## Abstract

We investigated the efficacy of Bt maize event MON810 against the spotted stem borers, *Chilo partellus* and *Busseola fusca* under artificial infestation in two plantings. The treatments comprised eight Bt hybrids (positive) and their non-Bt versions (negative), one conventional stemborer resistant maize line and three commercial checks grown in the country, laid out in an alpha lattice design, and replicated four times. Data were collected on all the stem borer damage parameters (leaf damage, number of exit holes, number of internodes tunneled, and length of tunnels) and yield.

The results derived from both plantings consistently showed significant differences between the Bt and non-Bt hybrids in all damage parameters and yield. The Bt gene significantly reduced leaf damage, numbers of exit holes and number of tunneled internodes, and length of tunnels after artificial infestation with stem borer neonates. As a result, the yield of Bt. hybrids was significantly higher than those of their negative versions, conventional stemborer resistant check and the commercial checks. These results have therefore demonstrated that the Bt. gene has a positive and significant effect on yield.

**Keywords**: *Busseola fusca, Chilo partellus,* MON810, Uganda

## Introduction

The most prevalent and damaging insect pests on maize in Uganda are the lepidopteran stem borers which include the spotted stem borer (*Chilo partellus* Swinhoe), African stemborer (*Busseola fusca* Fuller), the sugarcane borer (*Eldana saccharina* Walker), and the pink stem borer (*Sesamia calamistis* Hampson) (Kalule *et al*., 1994). *Busseola fusca* and *C. partellus* are the most abundant followed by *S. calamistis,* with few records of *E. saccharina* reported. Evidence on the ground shows that their distribution and pest status is changing in space and time (Kalule *et al*., 1997; Molo., 2014).

The stem borers feed on leaves, stems and maize cobs. Feeding by borer larvae in whorls of the maize plants usually leads to dead-heart and early leaf senescence. Feeding in the stem leads to reduced translocation of nutrients and assimilates. Stem tunneling also reduces plant vigor and the grain filling process, and promotes breakage of the plants as they mature. The feeding damage also extends to the cob causing substantial damage, including accumulation of aflatoxins in the grain. In Uganda, Kalule *et al*. (1994) reported that stem borers reduce maize production by up to 12-31%. These yield losses translate directly into food insecurity and financial losses to farmers. In addition, an estimated 30% of resources are wasted during production of the crop, including the land, labour, seeds and fertilizers. This loss can increase to 100% under severe stem borer infestation, particularly in drought occurrence.

The methods recommended for control of stem borers are the use of cultural practices (such as conservation agriculture using the Push-Pull system), biological control (using predators, pathogens and parasites), chemical pesticides and host plant resistance. However, each has its own limitation(s). Land limitation, knowledge and labor intensiveness limit the widespread adoption of Push Pull (Mukebezi, 2008). Insecticide use is not cost effective in smallholder conditions and exposes farmers to health risks and can result in environmental damage. Biological control involves skills and delay to reduce pest population. Many farmers, therefore, resign to not controlling stemborers.

In response to the mentioned limitations, NARO imported and sought NBC approval to evaluate MON810 for efficacy against the major stem borer species (*C. partelus* and *B. fusca*) in Uganda. Bt maize event MON810 carries a gene from the common soil bacteria *Bacillus thuringiensis* (Bt). The gene codes for the Cry1Ab protein, which is selective for lepidopterans (Gonzalez-Nuñez*et al*., 2000). Bt maize event MON810 was de-regulated and commercialized in the United States since 1996. It has been approved for import and cultivation in many countries in Latin America, Asia and Europe since 1998. In Africa, farmers in South Africa have planted Bt maize since 1998. By 2010, it was already clear that the benefits of biotechnology-derived insect resistance maize were evident based on the area planted to Bt maize expanding to greater than 40 million hectares (James, 2010).

**Objective**

The objective of the trial is to evaluate the performance of Bt maize event MON810 against *C. partellus* and *B. fusca*, the major stem borer species in Uganda.

**Materials and Methods**

The trial comprised 16 entries consisting of the Bt and non-Bt isogenic versions of eight hybrids and four checks. The four checks consisted of Namulonge developed varieties (Longe 6H, and Longe 10H) and a Monsanto commercial variety (DK 8031) and a stem borer conventional resistant hybrid (CKIR06009) from the International Center for maize and wheat research.

The trial was planted in an alpha lattice design, replicated four times. There were two row plots per entry. Two seeds were planted per hill in a row of five-meter lengths and thinned to one seedling per hill 2-weeks after emergence. The spacing was 75 cm x 30 cm, respectively. The field was kept weed-free by hand weeding. Five plants (2nd to 6th) from each row were infested with ten stem borer neonates (less than one week old), starting about 3 weeks after emergence and repeated at weekly intervals, for a total of three infestations.

Data were collected on stem borer damage parameters (leaf, stem and node damage) and yield. Leaf damage was scored on a scale of 1 – 9; where 1 = no visible leaf feeding damage; 2 = few pin holes on older leaves; 3 = several shot-holes injury on a few leaves; 4 = several shot-hole injuries common on several leaves or small lesions; 5 = elongated lesions (> 2 cm long) on a few leaves; 6 = elongated lesions on several leaves; 7 = several leaves with elongated lesions or tattering; 8 = most leaves with elongated lesions or severe tattering; 9 = plant dying as a result of foliar damage. The results were subjected to analysis of variance. The means of the three checks were pooled together.

**Results**

The results presented herein are for the two CFTs. The results for the CFTs were consistent for all the parameters collected. Thus, the presentation combines results of both, infested with the spotted stem borer.

**a. Grain yield**

Grain yield was significantly higher in the Bt than in the non-Bt maize (negatives, conventional resistant hybrid and commercial checks) (Fig. 1). When combined for the two trials, the mean yield of maize was 6.6 t/ha for Bt, 3.7 t/ha for the negative versions of the Bt, 4.8 t/ha for the conventional resistant hybrid and 3.4 t/ha for the commercial checks. These results showed that the yield of Bt maize was almost twice that of the commercial checks and the negatives checks under high stemborer pressure. Also, Bt maize out-yielded the conventional resistant hybrid by 1.4 times.

**b. Leaf damage**

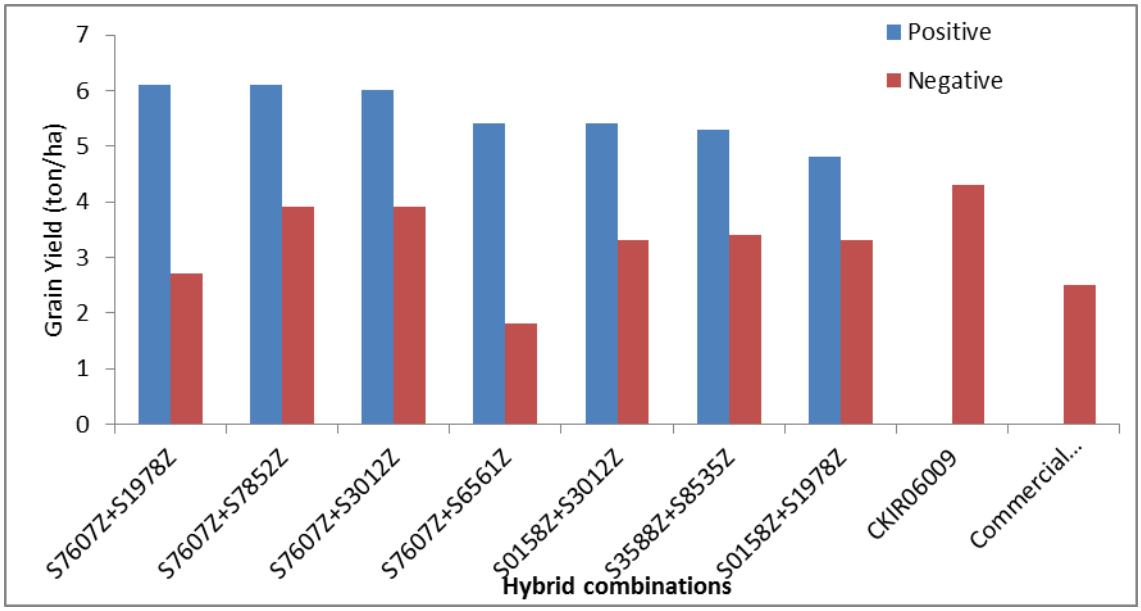
All the Bt lines were less damaged by stem borers when compared with the non-Bt. They were highly resistant to stem-borers, with a mean leaf damage score of 1.1. The conventional resistant check also succumbed to some extent of stem borer damage (score of 3.8). The negative versions of Bt hybrids also succumbed to leaf damage (score of 5.1). However, leaf damage was more pronounced in the commercial checks (score of 5.2).

### c. Number of exit holes

The number of exit holes was higher on the non-Bt than on Bt maize hybrids on both trials (Fig. 2). The number of exit holes averaged 0.2 for Bt, 5.9 for the negative versions of Bt, 3.4 on the conventional resistant hybrid and 5.9 on the commercial checks.

### d. Number of internodes tunneled and length of stem tunnels

The number of internodes tunneled was lower in the Bt hybrids when compared with the non-Bt hybrids (Figs. 3 and 4). The number of internodes tunneled averaged 0.1 in Bt, 2.5 in negative versions of Bt, 1.6 in the conventional resistant hybrid and 2.6 in the commercial checks. Similarly, the extent of stem tunneling was lowest in Bt (0.3 cm), compared with 3.6 cm in the negative versions of Bt, 3.8 in the conventional resistant hybrid and 4 cm in the commercial checks.



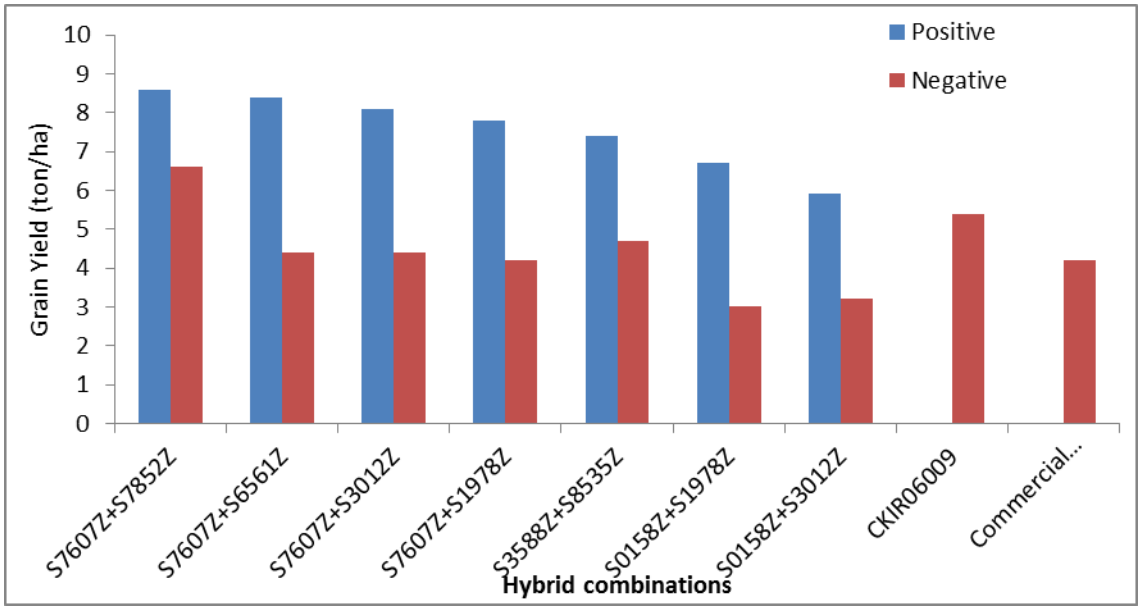
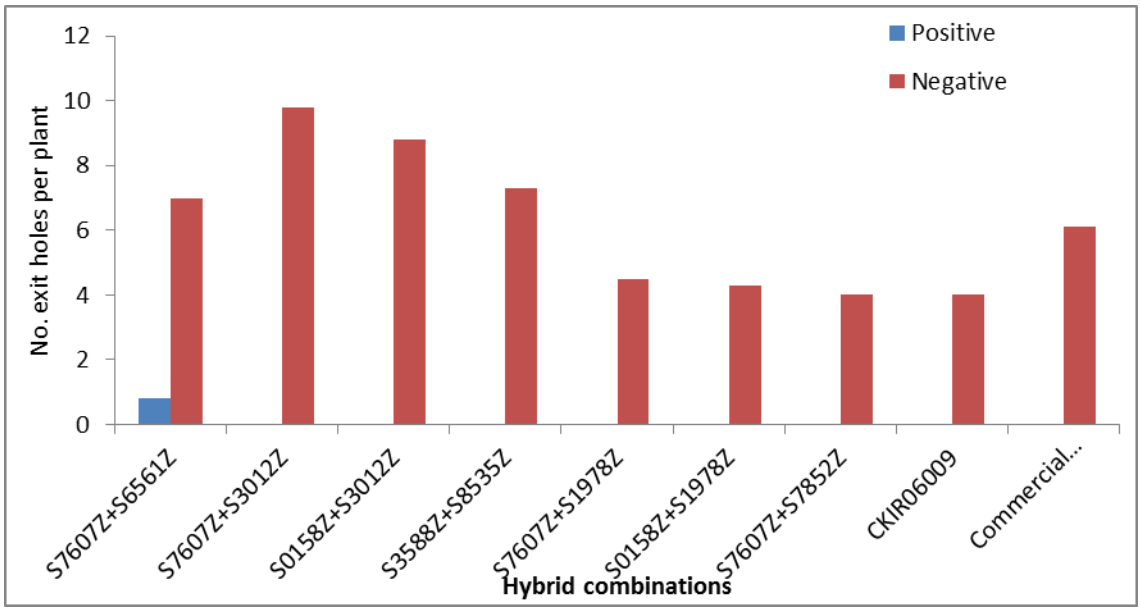



**Figure 1. Grain yield of different maize genotypes after artificial infestation with neonates of the spotted stemborer in the first planting (first graph) and second planting (second graph)**



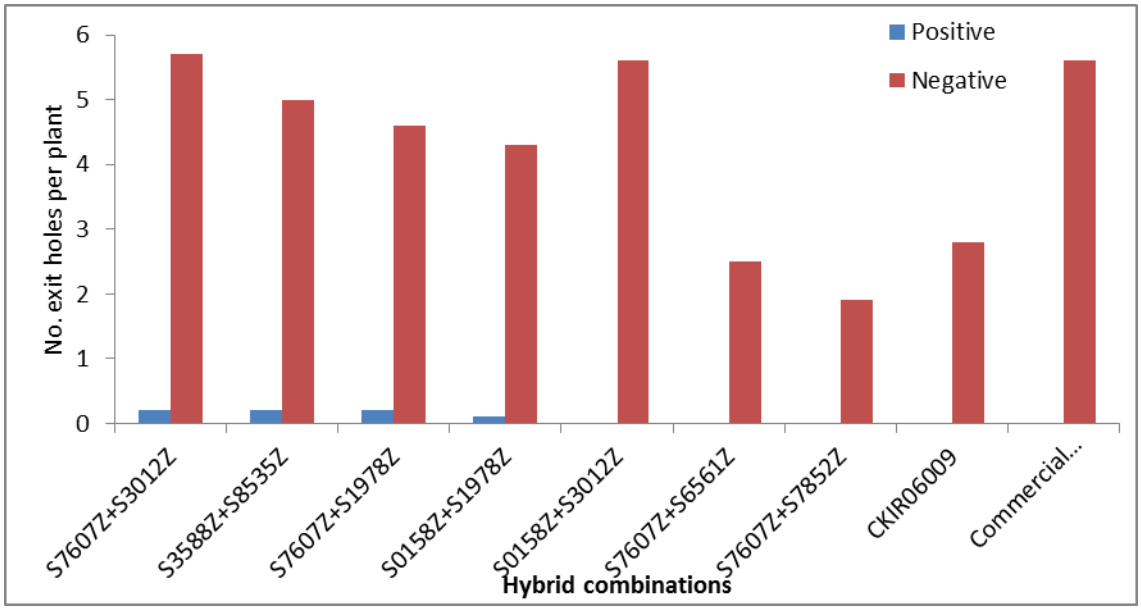
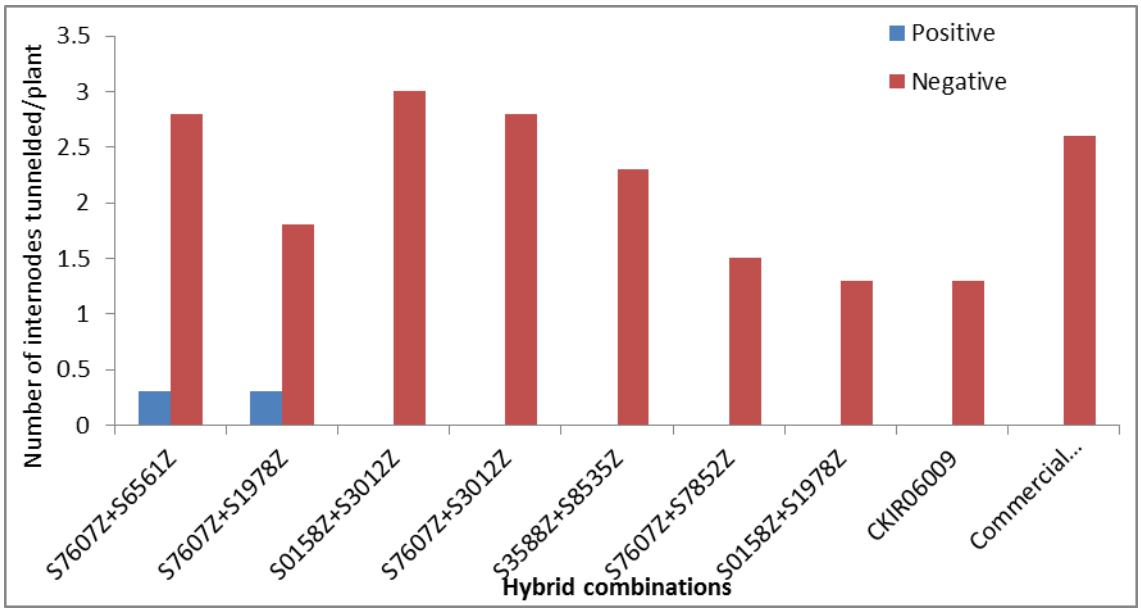



**Figure 2. Number of exit holes on different maize genotypes after artificial infestation with neonates of the spotted stemborer in the first planting (first graph) and second planting (second graph)**



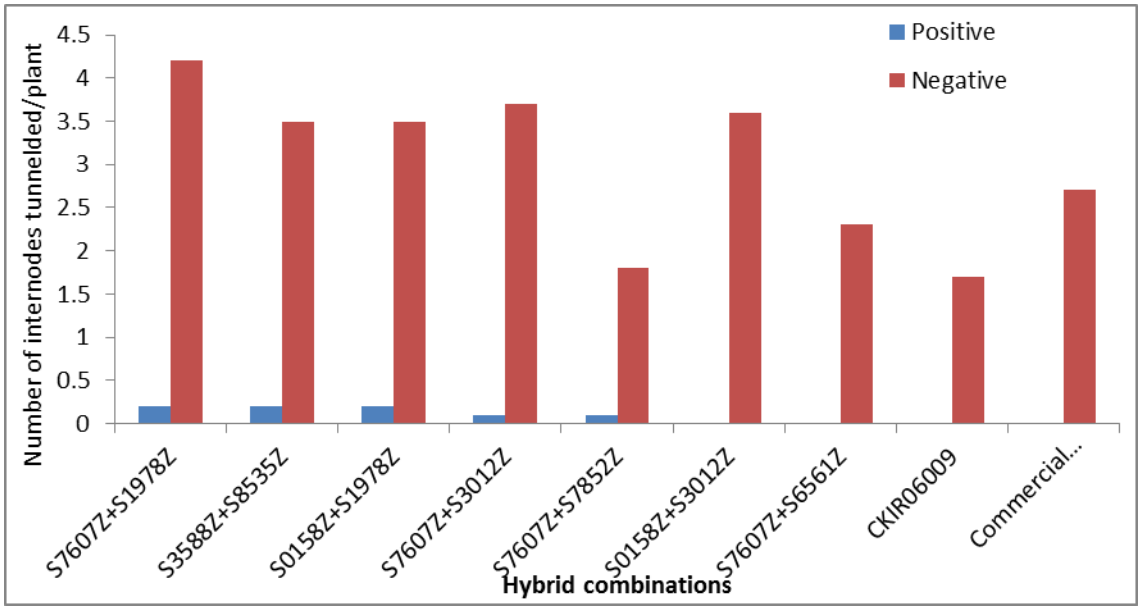
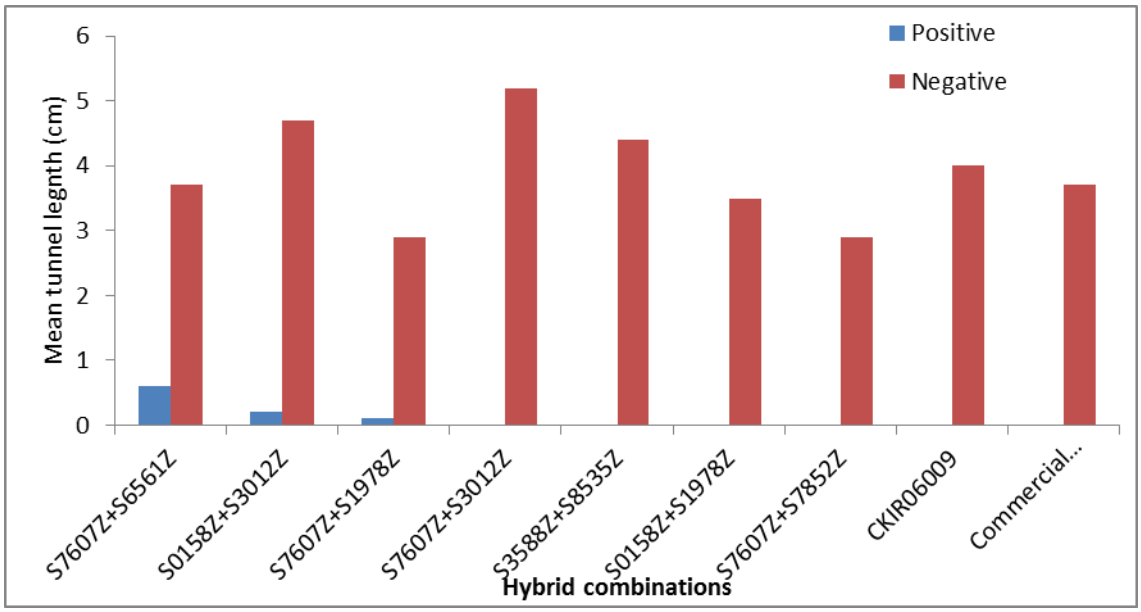
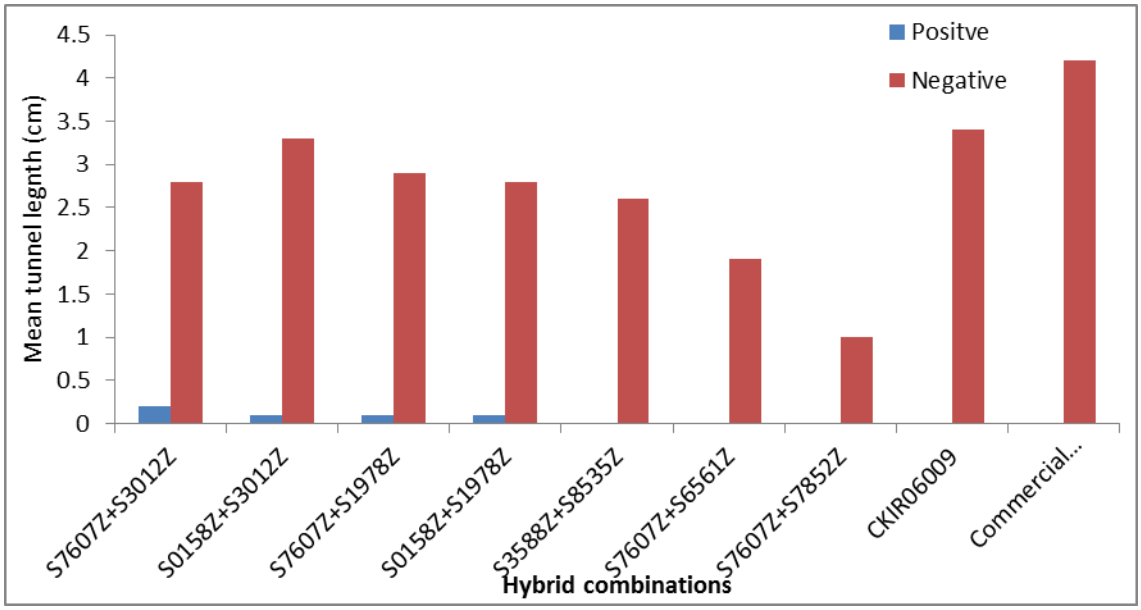



**Figure 3 Number of internodes tunneled on different maize genotypes after artificial infestation with neonates of the spotted stemborer in the first planting (first graph) and second planting (second graph)**






## Conclusion

Bt event is effective against the *C. partellus* and can help protect maize crop against the devastating effect of stem borers. Potentially, farmers could earn two million five hundred thousand Uganda shillings (at a farm gate price of 800 UGX per Kg) when using Bt maize instead of the susceptible varieties under severe stem borer infestation.

## Recommendations

Conduct multi-location trials under natural infestations to assess the response of stem borers in different agro-ecologies.


## Acknowledgement

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## 4.7 Assessment of effectiveness of Genetically Modified (GM) Cotton Variety (Bollgard II) to control bollworms

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**Abstract**

Bollworm damage to cotton was one of the concerns by stakeholders in the cotton industry. A study to test effectiveness of GM cotton in the control of bollworms under Uganda conditions was recommended. Bollgard II was tested under Confined Field Trials at two locations at the National Semi Arid Resources Research Institute (NaSARRI) in Eastern Uganda and at Mobuku Prison Farm, Kasese, in Western Uganda, for the first time during the cotton season of 2009/2010 and 2010/2011.

GM SG-125-BGII cotton was tested along with one other GM variety 06Z604B, their non-GM isolines SG-125-99M03 and BPA 2002, a Ugandan commercial variety. The experiment was carried out in a randomized complete block design with 10 treatments replicated four times.

In 2010/11 a stacked gene variety (SG125B2RF) for Roundup tolerance and bollworm control was introduced to replace GM variety 06Z604B. Each variety had a spray and no spray component. All treatments were sprayed with Golan insecticide 3 times at the rate of 24mls/16 liters of water against sucking pests.

At Serere low numbers of bollworms were recorded on GM varieties, SG-125-BGII and 06Z604B as compared to bollworm numbers on unsprayed variety BPA 2002 and non-GM isolines SG125 and 99M03.
In Kasese a similar trend of very low numbers of bollworms on GM varieties was recorded. The 3 genetically modified varieties SG-125-BGII, SG-125-BG2RF and 06Z604B appear to control bollworms feeding on them by killing the larvae.

**Keywords:** Stacked gene, Isoline, Bollgards, Confined Field Trials.

### Introduction

Cotton is an important strategic crop in the economy of Uganda. Introduced in 1903, it became a major foreign exchange earner. Its peak production was in the late 1960s to early 1970s when Uganda produced 465,000 bales of lint annually and it was contributing 40% to the country's foreign exchange earnings. Cotton is produced mainly by smallholder farmers and contributes to income of approximately 10% of the population. Ugandan cotton is of high quality with medium to long staple, which guarantees a stable demand at the international market.

Cotton production has declined since the late 1970s. Factors leading to the decline include political instability at the time, unaffordable costs of production inputs (e.g. insecticides, herbicides, fertilizers), and unreliable rainfall, and fluctuation of the cotton price. Loses due to the above constraints are estimated at around 100,000 bales of lint, an equivalent of US20 million in export value per annum.

Stakeholders have been concerned about the decline in production. Consultative meetings were held and it emerged that pests, mainly bollworms were the major constraint causing 40% loss, followed by weeds, causing up to 30% loss.

Current control measures were found inadequate. Genetically Modified (GM) cotton was considered as a viable option after considering its success in other countries. It was then recommended that NARO scientists test the efficacy of Bt cotton in controlling the damage of bollworms to cotton under Uganda's conditions.

Assessment of genetically modified cotton varieties to control bollworms was conducted in Serere and Kasese. Two genetically modified varieties, SG125BGII and 06Z604B were assessed and compared with non-genetically modified varieties SG125 and 990M03, and BPA 2002 a Ugandan commercial variety. The experimental design was a randomized complete block with 10 treatments replicated 4 times. Experimental plots measured 3.75 m x 12 m in Serere and 4.5 m x 12 m in Kasese. Insect/damage counts were conducted on 24 randomly selected plants, using the middle two data rows weekly from appearance of first squares until opening of first bolls.

In 2010, a stacked gene (SG125B2RF) for Roundup tolerance and bollworm control was introduced to replace GM variety 06Z604B. Each variety had a spray and no spray component. All treatments were sprayed with Golan insecticide (Acetamiprid 200g/L) three times at the rate of 24mls / 16 liters of water against sucking insect pests.

The treatments were kept weed free by hand hoeing. Data recorded showed aphids, jassids, and white flies, as common pests on cotton. Counts on natural enemies showed lacewings, ladybird beetles, *Orius* spp. and spiders to be more abundant in Serere and ladybird beetles and spiders in Kasese.

In both Serere and Kasese bollworm larvae counts were significantly lower on GM cotton varieties compared to unsprayed non-GM varieties and unsprayed BPA 2002.
Results of 2 seasons' data showed that genetically modified varieties SG-125-BII, SG-125-B2RF and 06Z604B do control bollworms on cotton by killing larvae and the stacked gene controls bollworms.

**Recommendations**
It is recommend that scientists conduct the trial for another season to verify the results obtained and that work on introgression of Bt gene into Uganda varieties be started.

**Acknowledgements**
The authors would like to express gratitude to the following: CDO, NARO, IBC, NBC. UNCST, MAAIF, PBS, IFPRI, ABSP II, APEP, MONSANTO, Stakeholders in the cotton Industry.

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## 4.8 Testing Roundup-ready Cotton in Uganda

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[2]*Mobuku Prison Farm, Department of Agriculture, Kasese district.*

**Abstract**
Roundup-ready cotton was tested under confined field trials at NaSARRI-Serere in Eastern Uganda and Mobuku Prison Farm-Kasese, Western Uganda during the 2009/2010 and 2010/2011 cotton seasons. Weeds were controlled using two dosages of Roundup. These were the recommended rate and twice the recommended rate applied to transgenic cotton. The recommended rate was applied at 220 mls /15 litres of water, while the double rate was applied at 440 mls/15 litres. For the BPA 2002 control commercial variety, weed control was done through hand weeding three times. Major weeds of cotton were fully controlled by herbicide application at the two rates applied twice on the transgenic cotton without any negative effect to the cotton crop. At NaSARRI during the second season, application of the recommended rate of roundup twice to variety SG 125RRFlEX gave seed cotton yields of 1015.6 kg/ha, compared to 1265.6 kg/ha when the rate was doubled. The local commercial variety BPA 2002 hand weeded three times during the same season yielded 1495.6 kg/ha. The results point to the prospective success of herbicide use on transgenic cotton under Ugandan conditions and strengthen the need for introgression of the technology to the local cotton varieties in the country.

***Key words****: Cotton, weeds, Transgenic, Roundup, Roundup-ready.*

**Introduction**
Weeds are a big problem to cotton production in Uganda. Up to 98 % of cotton farmers in Uganda weed cotton by hand, 1.5 % use tractor and ox drawn weeders at one time, while 0.5 % use herbicides. Studies conducted at NaSARRI revealed sharp increases in seed cotton yields, revenue, gross margins and costs with increased weeding frequency up to four times (Elobu *et al*., 1998). Beyond this frequency, the increases were low and gross margins fell.

The technology of transgenic herbicide tolerant (HT) plants has reduced the cost of weed control and boosted the production the production of several crops such as soya beans, maize, and cotton, in several countries. For the case of cotton, Monsanto developed a technology in which a gene picked from a bacterium (*Agrobacterium tumefaciens)* and inserted in the plant genome. The gene leads the plant to produce enzymes that make it resistant to glyphosate. Glyphosate is the active ingredient in Roundup, which is responsible for killing all susceptible plants to which the herbicide is sprayed.

The objective of this work was to assess the ability of Monsanto's genetically modified (GM) cotton varieties to tolerate applications of Roundup herbicide, as a weed control method under Uganda's conditions. The gene was later to be introgressed into Uganda's cotton varieties for commercial production after three years of confined field trials, if results were promising.

**Materials and methods**
Cotton was grown under confined field trials at the NaSARRI in Serere district and at Mobuku Government Prison Farm in Kasese district in 2009/2010 and 2010/2011 seasons. Fourteen (14) treatments were studied in the first season. They comprised of five cotton varieties, two

hand weeding and three herbicide application schedules. The five varieties were SG125RRF and DP110 which were transgenic and had the gene for resistance to roundup, their isolines (SG 125 and 99M03, respectively) and the non-transgenic Ugandan commercial variety (BPA 2002). The two hand weeding treatments were weeding six times to give 100 % weed-free situation throughout the season and Ugandan Farmers' practice of weeding three times. The three herbicide application schedules were 220 mls of Roundup/15 litres of water applied once over the top of cotton, 220 mls/15 litres applied twice over the top of cotton, and 440 mls/15 litres applied twice over the top of cotton)

In the second season, twelve treatments were studied and they were composed of: (a) four varieties *(*SG 125RRF with single gene for tolerance to Roundup herbicide, SG 125 B2RF with stack genes for Roundup tolerance and bollworm resistance, their isoline SG 125 and the local commercial BPA 2002 as the local control. (b) two hand weeding methods as in season one (c) three herbicide application schedules as described for the first season. Weed data was recorded before and after any herbicide application from two stations of 0.5 $m^2$ per plot. Sucking pests, especially aphids, white flies, and lygus, were controlled from all plots using Golan (Acetamiprid 200g/L) at a rate of 24 mls /15 litres of water while bollworms were controlled by sprays with Twiga Cyper at a rate of 120 mls /15 litres of water. At the end of each trial and after taking all the required data, all the cotton from the confined field trial sites was incinerated in the incineration pit within the facility. At harvest, cotton for yield data was picked from four middle rows leaving three stands on either ends of each row.

**Results and discussions**

There were big reductions in numbers of weed species counted a week after Roundup application, compared to numbers before. This was true for both broad-leafed weeds and grasses. The common ones at Mobuku were *Caccia occidentalis, Caccia obtusifolia, Portulaca quadrifida, Portulaca oleraceae, Solanum incanum, Commelina benghalensis, Allium* spp., and *Cyperus rotundus.* At NaSARRI, *Spermacoce latifolia, Commelina benghalensis, Cyperus rotundus, Eleochari scompanata, Paspalum* spp*, Panicum maximum* and *Digitaria scalarum* were the common weeds observed after spraying Roundup. At NaSARRI, the biggest reduction was caused by application of 440 mls/15 litres ,before Roundup application, numbers of both broad leafed weeds and grasses were not significantly different across treatments, as expected. Significant reductions occurred after herbicide application. At Mobuku, 220 mls/15 litres, especially on variety SG 125 RRFLEX, worked effectively like 440 mls/15 litres. The weeds observed after Roundup application, however, were weak, heading to die and could not offer any significant competition to the cotton crop.

Yields of the transgenic cotton varieties and their isolines at NaSARRI were not significantly different from BPA 2002 under all the weed management treatments. Weeds as a variable had no major impact on yields at NaSARRI suggesting that below a certain threshold of weeds, cotton performance was not affected. All the weed management treatments controlled weeds below that threshold. At Mobuku, however, all the "new" technologies out-yielded BPA 2002 under Farmers' practice. SG125RRF and its isoline SG125 performed better than DP110 and its isoline 99M03, which in turn performed better than the control, BPA 2002.

At NaSARRI during the second season, the stack gene variety (SG125B2RF) hand weeded six times significantly out yielded BPA 2002. When sprayed with Roundup once or twice, yields were not significantly different from the controls. The same observation was true for the other transgenic variety and both isolines. They exhibited no superiority over BPA 2002. At Mobuku, application of the recommended rate of Roundup twice, and double the recommended rate applied twice significantly increased yields of the transgenic varieties SG125RRFEX and SG125B2RF compared to the control BPA 2002 weeded three times and - six times.

These results point to the prospective success of herbicide use on transgenic cotton under Ugandan conditions and strengthen the need for introgression of the technology to the local cotton varieties in the country.

**Acknowledgements**
Special thanks go to Monsanto, USA who provided funds and the technologies for this study. The team on the ground was a big one and is highly appreciated. They include Dr. T.E.E Areke (Breeder and PI), Dr. George Epieru (Entomologist), Dr. Nelson Wanyera (Botanist), Mr. James Ronald Ocan (Scout, NaSARRI), Mr. Denis Ocen (Scout, NaSARRI), Mr. Ronald Kawooya (Scout, Mobuku), Mr. Martin Katende (Scout, Mobuku), Ms. Josephine Namugerwa (Scout, Mobuku). We also thank other partners that provided various technical and financial supports for this work. They include among others, NBC, Institutional Biosafety Committee (IBC) of NARO, Programme for Biosafety Systems (PBS), ABSP-II, and Science Foundation for Livelihoods and Development (SCIFODE).

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## 4.9 Transgenic bananas with resistance to banana nematodes and weevils

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**Abstract**
Banana parasitic nematodes cause severe root damage and subsequent plant toppling that often leads to 100% yield loss. Nematodes also persist in the soil for quite a long period and there is no effective cultural control of nematode after plantation establishment. Resistant varieties have the potential for sustainable and cost-effective farmer control option for the nematodes. The genetic engineering approach was utilized to develop transgenic banana lines of predominantly cultivar Sukali Ndizi expressing single or double combinations of antinematode transgenes (modified rice (*OcIΔD86*) cystatin, potato aspartic protease inhibitor (PDI) and nematode repellent peptide) driven by constitutive CaMV35S or maize ubiquitin promoters. Other genes used were CRY6A, papaya cystatin and the stack of two genes. The transgenic bananas expressing those transgenes were then evaluated at NARL, Kawanda under screen house and confined field trial conditions. All the biosafety requirements were strictly adhered to at all stages during development and evaluation of these transgenic banana lines. Transgenic lines were assessed and ranked in order of importance for the level of resistance at harvest and

its significance. Twelve 12 promising transgenic banana line selections expressing single or dual antinematode transgenes providing more than 90% resistance. These promising transgenic banana lines have been bulked and are being proposed for replanting in confirmatory second CFT. Additionally, over 40 lines of transgenic Gonja with CRY6A, papaya cystatin and their stack with over 90% protection against nematodes have identified under screenhouse conditions. Similary generated Gonja lines are under evaluation for weevil resistance and other Nakitembe transgenic lines are being prepared for inoculation with weevils and nematodes under screehouse conditions. Those lines with nematode resistance confirmed to be high are being bulked for CFTs. So far, technologies being used to develop transgenic nematode resistance in East African highland bananas are very promising.

Keywords: Transgenic banana, parasitic nematodes, confined field trial, transgene expression

**Introduction:**

Banana weevil and banana nematodes are two key pests that cause high yield losses in banana production in East and Central Africa. The population buildup of both pests is greatly delayed by establishing banana plantations with clean planting material, either with tissue culture plants or with pared suckers. However, there is no dependable cultural control on banana nematodes in established fields. It is possible to control banana weevils effectively in established by denying them favourable breeding conditions (dark and wet conditions). This, however, involves continuous digging up old corms and chopping them with other harvested pseudo-stems into small pieces, which is laborious. When the chopped pieces are spread to dry, all weevil lifecycle is disrupted as the eggs, the larvae and pupa are desiccated to dead.

Use of resistance for control of both banana weevils and nematodes is considered to be the most effective and sustainable. The genetic engineering approach was utilized to develop transgenic banana lines expressing antinematode transgenes. This abstract documents the current status of the development of nematode transgenic resistance to banana weevils and nematodes.

**Methods**

Genetic engineering was used to develop transgenic banana lines of cultivar Sukali Ndizi expressing single or double combinations of antinematode transgenes (modified rice (OcIΔD86) cystatin, potato aspartic protease inhibitor (PDI) and nematode repellent peptide) driven by constitutive CaMV35S or maize ubiquitin promoters. The transgenic bananas expressing those transgenes were then evaluated at NARL, Kawanda under screen house and confined field trial conditions. All the biosafety requirements were strictly adhered to at all stages during development and evaluation of these transgenic banana lines.

Embryogenic cell suspensions of Gonja and Nakitembe both more susceptible to banana weevil and nematode susceptible than Ndizi were transformed with CpCYSΔ89, Cry6A and a combination of the two genes. Fifty to ninety lines of Gonja per each gene construct of the generated lines that were confirmed to have the genes were established in pots and inoculated with nematodes and weevils. Other 240 lines of Nakitembe have been generated and being characterised by PCR in readiness for inoculation with weevils and nematodes.

**Results and Discussion:**

Transgenic lines were assessed and ranked in order of importance for the level of resistance at in a screen house and at harvest in the field. Twelve 12 promising transgenic banana line

selections were expressing single or dual antinematode transgenes and providing more than 90% resistance under field conditions. These promising transgenic banana lines have been bulked and are being proposed for replanting in confirmatory second CFT (application submitted for approval). Additionally, over 40 lines of transgenic Gonja with CRY6A, papaya cystatin and their stack with over 90% protection against nematodes have been identified under screen house conditions. Similary generated Gonja lines are under evaluation for weevil resistance and other Nakitembe transgenic lines are being prepared for inoculation with weevils and nematodes under screen house conditions. Those lines with nematode resistance confirmed to be high are being bulked for CFTs (CFT planned this year). So far, technologies being used to develop transgenic nematode resistance in East African highland bananas are very promising.

**Acknowledgements**

The authors would like to express gratitude to all partners from various Universities, International Research Centres that have provided scientific backup, the development partners for financial support and the regulators, IBC, NBC and MAAIF for supporting implementation of the activity.

## 4.10 Transgenic bananas with resistance to banana bacterial wilt

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**Abstract**

Banana bacterial wilt caused by *Xanthomonas campestris* results in 100% loss of infected bananas thereby threatening the livelihood of about 80% of farmers and economic loss of up to $2-8 billion over a decade in Uganda alone. There is no known natural resistance in the existing cultivated banana germplasm for the rapidly spreading destructive disease. In previous studies, disease resistance against bacterial wilt was confirmed in transgenic bananas of two cultivars "SukaliNdizi" (dessert banana) and Nakinyika (AAA-EA cooking bananas). Transgenic plants for the two cultivars, generated by agrobacterium mediated transformation constitutively expressing Hrap and Pflp genes derived from sweet pepper. Sixty five transgenic lines of SukaliNdizi were artificially infected with the bacteria *X. campestris* and evaluated in two crop cycles in a confined field trial at National Agricultural Research Laboratories Kawanda, Uganda beginning October 2010. Ten lines of SukaliNdizi showed 100% resistance, were true to type with regard to vegetative and reproductive growth parameters including flowering, yield performance in mother and ratoon crops compared to the controls. Subsequent studies embarked on testing the durability of the transgenic BXW-resistance in at least two banana varieties expressing the proven Hrap and Pflp genes as well as agronomic performance across multiple agro ecological locations in Uganda. Transgenic bananas lines for the two choice cultivars Hybrid M9 (a Matooke derived cooking Hybrid resistant to major bananas diseases and pests) and Nakitembe (a popular Matooke cooking variety), were generated through agrobaterium-mediated transformation and evaluated at screen house level. A number of lines showing 100% resistance have been selected for subsequent evaluation in multi-

locational confined field trials in 3 agro-ecological zones of L. Victoria basin, L. Albert region and Western farmlands.

**Keywords:** Banana, Bacterial wilt, confined field trial, transgenic, resistance

**Introduction**:
Banana bacterial wilt or banana Xanthomonas wilt (BXW) disease threatens the food security and livelihoods of millions of banana farmers, consumers and traders in Eastern and central African. BXW is caused by *X. campestris* pv musacearum and there is no known resistance against the disease in the existing banana germplasm to exploit conventional breeding approaches for resistance. Biotechnology approaches were used to generate resistance to BXW in banana at NARL-Kawanda by the National Banana Research Program in collaboration with IITA. This extended abstract summarizes current progress on BXW transgenic resistance development in Uganda.

**Methods**
Genes from sweet pepper were acquired from Academia Sinica, licenced to AATF and sub-licenced to IITA and NARO. They were then used to transform embryonic cell suspensions of cultivars Ndizi and Nakinyika to generate transgenic lines by Agrobacterium mediated procedures. Presence of transgenes were confirmed by molecular characterisation procedures mainly polymerase chain reaction (PCR) analysis. Potted transgenic plants were evaluated under confined screen house conditions in pot assays where plants were subjected to controlled artificial inoculation with the Xanthomonas. Resistant lines were advanced to the confined filed trials (CFTs). The first CFT of GM banana resistant to Xanthomonas wilt was conducted in a single location at NARL, Kawanda beginning in October 2010. Sixty five transgenic banana lines that had showed resistance to BXW in screen house pot trials were planted for evaluation in this first CFT. Under the CFT conditions, transgenic lines were evaluated for BXW resistance through artificial inoculation with *X. campestris* pv. musacearum mimicking the procedure farmers infect their banana plants in the field. The first CFT involved evaluation of the mother plant out of tissue culture plus the first as well as second ratoon (daughter and granddaughter) plant of each line. Following similar procedures the two genes and their stack version were used to generate BXW transgenic lines of matooke hybrid (M09) and a local matooke (Nakitembe). These are currently at screen house evaluation stages awaiting clearance to establish a CFT in 2 agro-ecologies.

**Results and Discussion:**
At the end the first CFT, 10 transgenic lines consistently showed 100% resistance to BXW throughout the three successive crop cycles. Additionally, all the transgenic lines were true to type with regard to pre-flowering, flowering and yield characteristics compared to non-transgenic controls. The best 10 transgenic banana line selections from the first CFT were advanced for replanting in a second CFT to evaluate durability of disease resistance and agronomic performance at the same location at NARL, Kawanda. All the nine transgenic dessert banana Sukali Ndizi lines evaluated showed 100% disease resistance with both mother and first ratoon crop in comparison to non-transgenic plants. The perfect efficacy of transgenic banana expressing Hrap and Pflp genes against BXW provides evidence for possibility of using GM banana as control option.

Many transgenic lines of M09 and Nakitembe have been generated using procedures already described. In pot experiments under screen house conditions, the lines were inoculated twice with Xanthomonas. Lines that consistently showed 100% resistance (Figure 1) to BXW have been selected. The selected lines are being bulked in preparation for multi locational CFTs in Kawanda, Mbarara and Bulindi. Both Nakitembe and Hybrid M9 are susceptible to BXW and

popular among the farmers and traders due to its excellent bunch and cooking qualities. Hybrid M9 (Kabana 6H; Kiwanganzi), recently released by NARO's breeding team, is also resistant to black Sigatoka (*Mycosphaerella fijiensis*), tolerance to banana weevils, tolerant to parasitic banana nematodes which can cause up to 100% yield losses in susceptible banana varieties.

**Conclusion:**

Proof of concept studies showed that Hrap and Pflp genes singly confer 100% banana resistance to banana Xanthomonas wilt (BXW). Mulilocational CFTs will allow selection of BXW-resistant GM banana that have potential for future event deregulation and release for farmer growing in Uganda.

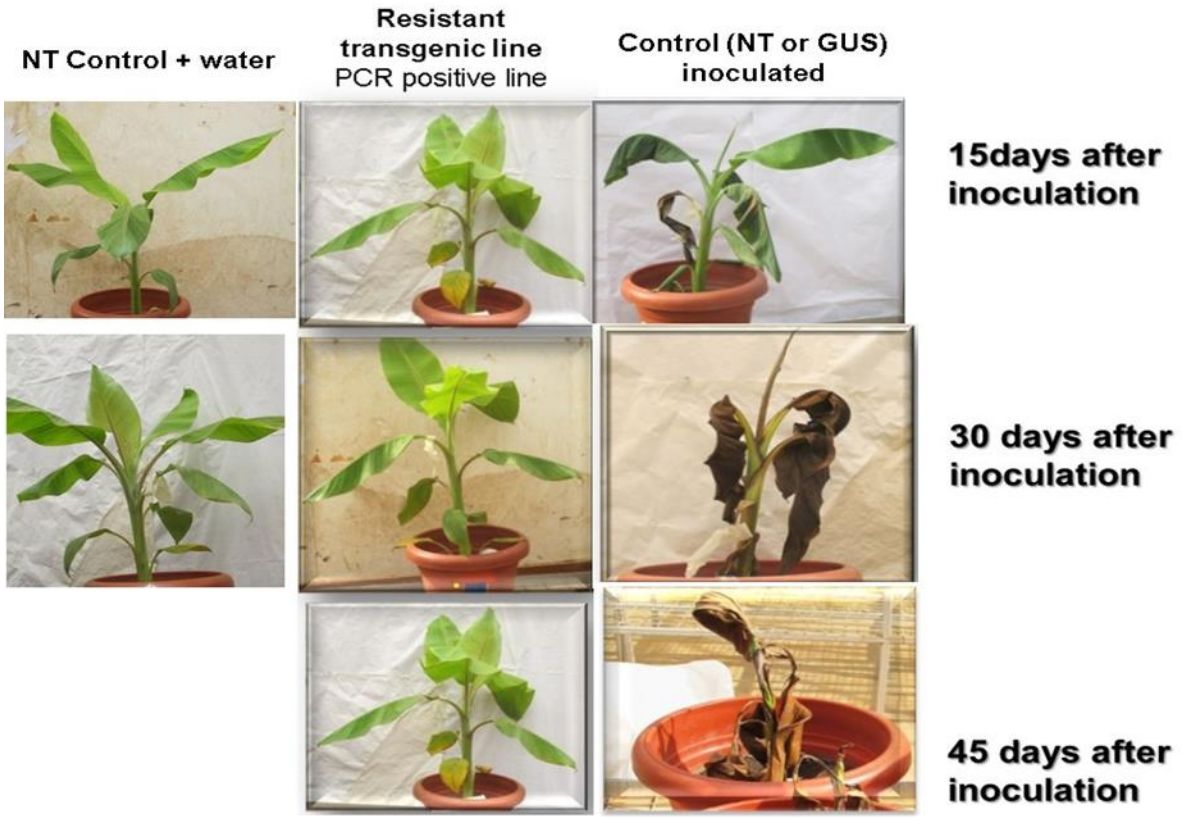


Figure 2: BXW disease development on M9 plants during screenhouse pot evaluation trials. BXW-resistant transgenic plants (Middle) show 100% resistance 40 days after first and second inoculation. Non-transgenic plants (Left) are completely wilted by end of 30 days after first inoculation.

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## 4.11 Development of transgenic bananas with enhanced Pro-vitamin A carotenoids

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**Abstract**
Despite consistent application of vitamin A supplements and the promotion of pro-vitamin A foods, Vitamin A deficiency (VAD) remains a major public health problem in Uganda with the percentage of children under 60 months ranging from 12.3% to 32.3% and women from 12.7% to 31.2% depending on region. We opted to increase Pro-vitamin A in the bananas that are already being eaten by millions of Ugandans, including the vulnerable children under 5 years and women of the reproductive age. This could be achieved through introgression of genes for enhancing Pro-vitamin A into bananas using conventional breeding. However, the majority of banana cultivars currently grown are essentially sterile or have extremely low fertility. This project, therefore, opted for use genetic engineering. Transgenic lines were generated using Apsy2a genes derived from high PVA banana cultivar Asupina. The first proof of concept CFT of GM banana with enhanced ProvitaminA (PVA) was conducted in at NARL, Kawanda since 2010. A follow-on CFT (product development stage) was established late 2014 again at NARL with cv. M09 and cv. Nakitembe transgenic with genes for PVA enhancement. Although we initially aimed at developing a transgenic line with 4 times PVA enhancement, up to 8 times pVAcarotenoid enhancement was obtained in M9 at proof of concept. In the current CFT (product development), a few bunches of M9 transgenic plants, so far harvested, have a striking orange colour characteristic of beta carotene compared to the control. Analysis for actual levels of beta-carotene will be confirmed by HPLC. The transgenic plants are true to type and there is no penalty in bunch weight. Technology so far shows promise for delivering an East African Highland banana with levels of PVA that are adequate to address the dreadful VAD in millions of Ugandan vulnerable children and women.

***Key words:*** *East African highland bananas, Biofortification, Provitamin A.*

**Introduction:**
Uganda carries a heavy public health burden with high levels of infectious diseases, primarily HIV and malaria, and very high levels of inadequate nutrition. Vitamin A deficiency (VAD) remains a major public health problem in Uganda with the percentage of children under 60 months ranging from 12.3% to 32.3% and women from 12.7% to 31.2% depending on region. This is despite the consistent application of vitamin A supplements and the promotion of pro-vitamin A foods.
One option for alleviating this nutritional burden is to biofortify staple crops, such as bananas, that already being eaten by millions of Ugandans at a daily basis but are low in pro-vitamin A and iron. This could be achieved through introgression of genes for enhancing Pro-vitamin A into bananas using conventional breeding or genetic engineering. However, the majority of banana cultivars currently grown are essentially sterile or have extremely low fertility. This project therefore opted for use genetic engineering to develop transgenic bananas with enhanced Pro-vitamin A. This abstract will give the status of implementation of this project.

**Methods**
All transgenic lines were generated by Agrobacterium mediated transformation procedures using Apsy2a genes derived from high pVA banana cultivar Asupina. Presence of trangenes were confirmed by molecular characterization procedures mainly polymerase chain reaction (PCR) analysis. Integration of genes into the banana genome and copy number were confirmed by southern hybridisation. The first proof of concept CFT of GM banana with enhanced

ProvitaminA (PVA) was conducted in at NARL, Kawanda since 2010. A follow on CFT was established late 2014 again at NARL with cv. M09 and cv. Nakitembe transgenic with genes for PVA enhancement. Data collected in the trials included agronomic data to ensure that the banana selected with enhanced PVA also true to type. Other data include bunch weight and PVA levels determined using HPLC. All regulatory guidelines are followed to the letter, with the support of Inspectors from UNCST and MAAIF.

**Results and Discussion**

The project aimed at obtaining a transgenic line with 4 times PVA enhancement. During the proof of concept, up to 8 times pVA carotenoid enhancement was obtained in M9. This then set a basis for the product development stage. In the current CFT, a few bunches of M9 transgenic plants have a striking orange colour characteristic of beta carotene compared to the control. Analysis for actual levels of beta carotene will be confirmed by HPLC confirms. The transgenic plants are true to type and there is no penalty in bunch weight.

**Conclusion**

Results from the proof of concept stage and preliminary results show that the technology is promising. Activities continue to aid identification of a good product for eventual deployment to address the huge nutritional deficiency problem in Uganda.

**Acknowledgements**

The authors would like to express gratitude to Queensland University of Technology, Australia for scientific backstopping, BMGF for financial support and the regulators, both IBC, NBC and MAAIF for supporting implementation of the activity.

## 4.12 Efficacy of MON 87460 Event in Conferring Drought Tolerance to Maize in Confined Field Trials in Uganda

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**Abstract**

Drought is a major abiotic constraint affecting maize production in Eastern and Southern Africa. Increased frequency of drought have resulted crop failures leading to famine because maize is a main food and feed crop. The sensitivity of maize to drought stress at critical periods during growing season discourages small-scale farmers from risking investment in best management practices, including use of improved seed and fertilizer. In fact, less than 10% of maize in Uganda is produced using best management practices. Water Efficient Maize for Africa (WEMA) public-private partnership was formed to develop and deploy royalty-free drought-tolerant and insect-pest protected white maize hybrid varieties to farmers. It was envisaged that increase maize yield stability under stress conditions, and protection from insect pests will promote farmers' investment in adopting best management practices. The WEMA drought tolerant varieties were expected to increase yields by 20–35% under moderate drought

over baseline varieties of 2008 varieties. The benefit of the transgenes will be combined with the benefits of conventional drought tolerant adapted to the tropical conditions in target countries.

A total of five confined field trials (CFTs) were conducted to evaluate the drought tolerant event MON87460 in Mubuku Irrigation and Settlement Scheme, Kasese following regulatory approvals. Similar trials were conducted in other participating countries of Kenya and Republic of South African. These confined field trials were conducted under managed drought conditions using established drought screening protocols. Our results from testing the transgenic MON87460 hybrids in CFTs showed a yield advantage up to 14% greater yield than the commercial hybrids checks under optimal conditions. One hybrid tested under managed drought stress had a yield advantage of up to 28.2% over the best commercial hybrid check, and did not suffer any yield penalty under optimum moisture conditions. The best hybrids were consistent in similar trials in other countries conducting the same trials. The test drought gene has positive and significant effect under drought without significant yield penalty under optimum-moisture condition under varying levels of drought stress.

*Key words**: **Drought tolerant maize, confined field trials yield advantage***

## 4.13 Genetically modified potato expressing resistant genes; *RB, Rpi-vnt1.1* and *Rpi-blb2* show high resistance to late blight disease in a confined field trial in Uganda

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**Abstract**
Late blight of potato, caused by *Phytophthora infestans*, is one of the most devastating diseases of potato (*Solanum tuberosum*L.) in Uganda and in many parts of the world. Conventional cross breeding to develop resistance has met with challenges of durability of resistance in the hybrids developed as well as more aggressive pathogen strains with resistance to fungicides. Three Resistance (R) genes; *RB*, *Rpi-blb2* and *Rpi-vnt1.1* previously cloned from two wild potatoes; *Solanum bulbocastanum* and *S. venturii*and the availability of a potato transformation system offered an opportunity to introduce all three genes into cultivated varieties in order to develop durable resistance. The genes were introduced into two cultivated potato varieties "Desiree" and "Victoria" at the International Potato Centre (CIP) using genetic engineering. The GM potatoes generated have been tested extensively in biosafety greenhouses in Peru, and recently in the field at Kachwekano Zonal Agricultural Research and Development Institute (KaZARDI) in Kabale Uganda. The field evaluation took place within a confined field trial approved and monitored by the National Biosafety Committee of Uganda. Twelve transgenic events of variety Desiree and one of variety Victoria were tested in three replications each consisting of randomized complete block design (RCBD) of plots of 15 plants each. The results indicate that 13 transgenic events are completely resistant to late blight disease with a significantly better yield performance compared to non-transgenic controls and current commercial varieties. These GM potatoes if incorporated in the seed potato system for uptake

by the farmers in Africa and elsewhere offer a great opportunity for durable cost effective and environmentally friendly solution to the management of late blight disease in potato. Therefore, further development and commercialization of these transgenic lines should be enhanced to avail farmers with late blight disease resistant varieties and reduce use of chemical control.

***Key words**: GM potatoes, transformation, late blight resistance, Phytophthora infestans*

## 4.14 Field resistance to East African cassava mosaic virus across three cropping cycles imparted by RNAi technology

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**Introduction**

Cassava (*Manihot esculenta Crantz*) is the major source of food for approximately 300 million people in sub-Saharan Africa (Nweke, 1996). Its cultivation is adversly affected by the two important virus diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) (Legg et al., 2011). CMD is caused by bipartite viruses possessing DNA-A and DNA-B components belonging to the family *Geminiviridea*, Genus *Begomovirus*, refered to as cassava mosaic geminiviruses (CMGs) and transmitted by transmitted by the whitefly vector, *Bemisia tabaci*. In East Africa CMD is caused predominantly by the two species, *African cassava mosaic virus* (ACMV) (Harrison et al.,1997; Pita et al., 2001) and *East African cassava mosaic virus* (EACMV) (Zhou et al., 1997), with the recombinant variant *East African cassava mosaic virus-Ugandan* (EACMV-UG), widespread in that region.

Post-transcriptional gene silencing (PTGS), also refered to as RNA interference (RNAi) is a technology for stable integration of traits such as resistance to virus disease (Duan*et al*., 2012). RNAi is based on an innate sequence-specific RNA degradation mechanism triggered by double-stranded (ds) RNA. Integration into the plant genome of an inverted repeat sequence derived from the targeted virus is used to initiate production of desired dsRNA. Inherent plant systems recognise the dsRNA and cleave it into 21–25 nucleotide small interfering RNAs (siRNAs) in the presence of Dicer proteins (Ding and Voinnet, 2007; Ruiz-Ferrer and Voinnet, 2009). One strand of the siRNA becomes incorporated into the RNA induced silencing complex (RISC) and acts as a guide to target the homologous viral sequences for degradation.

RNAi approaches has been successfully used to control plant viruses at the experimetal level and to generate genetically improved crops. Examplesinclude *Papaya ringspot virus* (PRSV) in papaya(Krubphachaya *et al*., 2007) and *Plum pox virus* (PPV) in plum(Hily *et al*., 2004; Kundu *et al*., 2008). Transgenically imparted geminivirus resistance has been reported in a range of crop species (reviewed by Shepherd et al. 2000) including beans (Aragao and Faria 2009), tomatoes (Fuentles et al., 2006), maize (Shepard et al. 2007) and in cassava against ACMV (Zhang 2005; Vanderschuren et al., 2007b; Chellepan 2004) and against ACVM and EACMV (Chellepan 2004; Taylor et. Al., this issue) within contained growth facilities.

The goal of the present study was to develop and test the efficacy of RNAi-mediated transgenic technology for controlling CMD in the field under conditions of high disease pressure. We describe here the performance of plants carrying inverted repeat sequences of the full length

and C-terminal of EACMV-UG *AC1* when grown in confined field trials over three cropping cycles. While transgenic plants became infected with ACMV, presence of EACMV remained undetected in the majority of the transgenic lines. Transgenic plants grew more vigorously than the controls and produced enhanced storage root yields. Data presented demonstrates the potential of RNAi technology to impart high levels of resistance to CMGs and supress the impact of CMD within these plants.

## Materials and methods

### Production and molecular analysis of transgenic cassava

Production and molecular analysis of transgenic plants of cultivar 60444 followed procedures of Taylor *et al*. (2012). Plants transgenic for 1-2 copies of the T-DNA and accumulating siRNAs specific to the RNAi constructs p560 and p561 (full length AC1 and C-terminal sequences of EACMV-Ug respectively) as determined by Northern blot analysis, were selected for testing under field conditions

### Plant acclimatization and field establishment

In vitro plantlets transgenic for p560 and p561 were established as individual micro-cuttings in 50 ml Falcon tubes (Ogwok*et al.,* 2012). A total of 640 plantlets (80 per line) were weaned within a Biosafety Level 2 screenhouse at National Crops Resources Research Institute (NaCRRI) for 8 weeks. Confined field sites were prepared by ploughing twice with a tractor three weeks prior to planting. Stem cuttings of the local cassava cultivar Ebwanateraka confirmed by PCR to be infected with EACMV were planted as infector rows around each plot in a 0.28 Ha field (50 m × 56 m). A total of 480 hardened cassava plants (60 per line) of transgenic 60444, non-transgenic controls of cv. 60444 and the CMD tolerant cv TMS30572 were used to establish confined field trials at NaCRRI, Namulonge, Uganda on October 14$^{th}$, 2009 (CMDCFT1) and October 28$^{th}$, 2010 (CMDCFT2). Plants were established in a randomized complete block design at a spacing of 1m × 1m (10,000 plants/ha), with each plot of 20 plants (5 × 4) replicated three times.

Stems cuttings from plants showing very mild or no CMD symptoms were preserved at the end of 2009 season (CMDCFT1) from the two most promising transgenic lines (lines 10 and 12 of p561) and non-transgenic controls of cv. 60444 and TMS30572. Stems cuttings measuring at least 10 cm in length were planted to establish a first vegetative cycle on October 8$^{th}$, 2010 with a plot size of 18 plants (6 × 3). At termination of this trial, stem cuttings were again preserved following the same criterion and used to establish a second vegetative cycle within the same trial site on October 11$^{th}$, 2011. Experimental design for the stake-derived plantings was maintained with the same number of replications and spacing as for tissue culture derived plants, but with a plot size of 30 plants (6 × 5).

### Data collection and analysis

Plants were assessed visually for CMD and CBSD incidence and severity on a weekly basis for 20 weeks, then bi-weekly until harvest of CMDCFT1 (2009-2010 season); bi-weekly during CMD CFT2 (2010-2011 season) season and monthly during vegetative stake generated CFTs (CMD DR1 and CMDDR2) until harvesting at 11-12 months after planting. All plants within each plot were scored on a 1–5 scale for CMD symptom severity (Terry, 1975). Numbers of adult whiteflies (*Bemisia tabaci*) were counted on the underside of the top five fully opened leaves. All inflorecences were counted and removed at least twice per week before anthesis to ensure reproductive isolation.

At harvest the innermost plants within each plot of CFTCMD1 and CFTCMD2 and for CMDDR1 and CMDDR2 were harvested by digging with a hand hoe to remove storage roots

from the soil. Data collected were analysed using ARM8 (formerly called Agriculture Research Manager software, Gylling Data Management, Inc., Brookings,SD, USA) for analysis of variance (ANOVA), and entry means were separated using Duncan's new multiple range test ($P = 0.05$).

**Sample collection and PCR detection of ACMV and EACMV**

The youngest CMD symptomatic leaf or equivalent samples were collected from plants of both transgenic and non-transgenic cassava lines at three different time points during the course of each trial. Total DNA was extracted from the leaf samples following the Dellaporta method (Dellaporta*et al*. 1983) and DNA subjected to PCR.

**Results**

**CMD disease development and severity**

CMD incidence reached maximum levels in CMD CFT1a 6-8 months after planting, with all transgenic and non-transgenic lines in the 60444 background reaching 100% symptomatic plants by this time (Table 1). No difference was observed for CMD incidence between control and transgenic lines in CMD CFT1, however, the following year in CMDCFT2 a significant difference in CMD incidence (P=0.002) was recorded (Table 2). In the latter case, a distinction was observed between the non-transgenic 60444 control and the AC1 RNAi lines, with the control plants becoming infected at a faster rate than the transgenics to reach 100% incidence by 7 MAP. In CMD CFT2, two transgenic events showed fewer than 90% symptomatic plants by 5 MAP, with 35% of the plants from transgenic line 561-11 remaining symptom free at harvest.

CMD severity was greater in CMD CFT1a compared to CMD CFT1b (Tables 1 and 2). In both field trials a significant difference was observed for severity of CMD symptoms (P<0.001) between the 60444 non-transgenic control and the transgenically modified lines. In CMD CFT1a the 60444 controls reached an average severity of 4.5-5, while all transgenic events developed a more moderate level of symptom development of between 2.5 and 3.5. Within CMD CFT1b this difference was more distinct with transgenic lines averaging CMD severities of 2.0-2.5, values similar to that of the CMD resistant cultivar TMS30572.

**PCR based detection of CMGs**

CMGs were PCR-detectible in 91.9% (328 out of the 357) of the samples collected from CMD symptomatic plants in CMD CFT1a (Table 3). All 278 transgenic plants in CMDCFT1 were confirmed, to be infected with ACMV, indicating that whitefly transmission of CMGs was highly efficacious. Presence of EACMV in this trial was detected in 71.4% of the non-transgenic cv. 60444 plants (Table 3), with 57.1% found to be dual infected with EACMV and ACMV. In contrast, EACMV was detected in less than 1.1% of the 278 leaf samples obtained from transgenic plants in the same field. A very similar situation was observed in CMDCFT2, in which all 161 transgenic plants tested were found to be free of detectible EACMV, but leaves from 100% of these plants were PCR positive for presence of ACMV. In CMD CFT1b 38% of the non-transgenic controls were seen to be infected with EACMV compared to 71.4% in CMD CFT1a (Table 4).

**Stability of RNAi imparted resistance**

Farmers do not plant tissue culture-derived plantlets, but propagate cassava vegetatively from stem cuttings. It was important, therefore, to assess whether the RNAi imparted field resistance seen in the CFTs described above would be durable across multiple vegetative propagation cycles. A field trial (CMD DR1) was established in October 2010 using 11 month old stem cuttings derived from plants transgenic lines 561-013 and 561-021 grown in CMDCFT1.

Stakes of cv. 60444 and TMS30572 were also carried over from CMD CFT1a and planted as controls. Twelve months later, plants from this trial were propagated for a third cropping cycle by planting stakes to establish field trial CMD DR2 in October, 2011

As expected, cuttings in CMDDR1 and CMDDR2 sprouted and immediately showed strong CMD symptoms on their young leaves. CMD incidence reached 100% of these plants within one MAP with the exception of TMS30572 in CMDDR2, in which case CMD was initially seen on 50% of the plants with new disease symptoms developing on a further 40% over the following eight months. In both CMDDR1 and CMDDR2, transgenic and 60444 control lines reacted significantly differently with respect to CMD severity ($P<0.001$). Non-transgenic 60444 plants developed very severe CMD symptoms, reaching maximum possible scores of 5 by 7-8 MAP. Conversely, the transgenic lines maintained a moderate disease score of 2-3 across the 11-month growing cycles of the two consecutive cropping seasons).

In order to determine the continued effectiveness of the imparted transgenic resistance to EACMV-UG over vegetative propation cycles, we deliberately planted stem cuttings of two transgenic lines of P 561 and non-transgenic controls from the original trial of 2009 and conducted two vegetative propagation cycles of cassava to mimic farmers' practices. The performance of the two transgenic lines compared to controls was quite similar to that observed during the 2009 and 2010 seasons. The transgenic lines carried very high incidence of single ACMV infection, but had no PCR detectible levels of EACMV-UG (Tables 5 and 6), compared to cv. 60444. This indicates that the imparted transgenic resistance against EACMV-UG remains strong up to the second vegetative propagation cycle.

**Discussion**

Confined field trials were established between 2009 and 2011 to determine efficacy of RNAi constructs for imparting resistance to CMD. Transgenic plants expressing siRNAs derived from the two inverted repeat genetic constructs of the full length AC1 (p560) and C-terminal AC1 (p561) of EACMV-Ug were tested under regulated field trials in Uganda. Plants transgenic for both p560 and p561 performed consistantly to show high levels of resistance to infection with EACMV with differences compared to the controls highly signficant in all four field trials (Tables 1 and 2). Almost complete exclusion of EACMV was acheived by the transgenic plants across the two trials established with tissue culture derived plants, while the non-transgenic controls were found to be infected with this pathogen at 71.4 and 38.5% respectively.

Performance of cassava transgenic for p561 (C-Terminal AC1) over consecutive stem propagations, demonstrated that the AC1-derived RNAi technology tested here was efficacious across vegetative cycles. While 60444 controls, and the conventional CMD-resistant cultivar TME30572, became co-infected with ACMV and EACMV at 100% and 68% respectively by the end of the third planting season, transgenic plants reached a maximum of less than 10% infection with EACMV over the same period.

This is the first report of RNAi-mediated control of EACMV-UG establishment and replication in field grown transgenic cassava up to the second vegetative propagation. In previous reports of hairpin mediated geminivirus control under controlled condition, only a small proportion of the transgenic lines would show resistance to the targeted geminivirus (Fuentes *et al*., 2006, Vanderschuren *et al*., 2007b). Overall these results provide evidence that the transgene was stably integrated, and expressed in the transformed lines throughout the two seasons, and provided high levels of resistance to EACMV-UG. Thus, the lack of detectable EACMV-UG in plants transgenic for P561 after two vegetative cycles clearly demonstrates the efficacy of RNAi technology to suppress infection and disease establishment of the targeted EACMV-UG across multiple planting cycles.

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## 4.15 Field Evaluation of Transgenic Sweet potato Lines Expressing siRNAs for resistance to Sweetpotato Virus Disease

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**Abstract**
Sweetpotato Virus disease (SPVD) caused by dual infection of mainly sweetpotatochlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV) is the most important disease constraint affecting sweetpotato in Uganda. Twenty-one sweetpotato transgenic events were evaluated for SPVD resistance in a confined field trial (CFT) at Namulonge. The main objective was to test whether RNA-silencing mediated resistance to the viruses is able to reduce spread of SPVD in the field. Three non-transgenic controls Huachano (susceptible), Ejumula (susceptible) NASPOT 11 (resistant) together with the events were planted in a complete randomized block design with 3 replications; each plot had 20 plants on two ridges of ten plants each; spacing was 1m between rows and 0.3m within rows. Each plot was surrounded by virus infected spreader rows of Ejumula to enhance the virus inoculum. Two subsequent crops; the second planted with "clean" cuttings from the first crop were evaluated. SPVD incidence and severity were recorded fortnightly starting with 1 month after planting (MAP) to 1 month before harvest. Incidence was captured as number of diseased plants/plot while SPVD severity was scored on a scale of 1-9 where 1=no visible symptoms, and 9=very severe symptoms. During the first planting, the whitefly population was low and hence SPVD symptom development was slow until scoring date 5. By the last date of scoring, the resistant check, NASPOT 11 had an incidence of 8.9% while the susceptible check Ejumula had an incidence of 50.9; Hachauno had the highest incidence (100%). During the second season, at scoring date 1, clones 4-C127-27, 9-C127-Y21, Ejumula, NASPOT 11, 7-C127-Y10 had SPVD incidences below 40%. However, by scoring date 5, only 7-C127-Y10 and NASPOT 11 had SPVD incidences below 60% suggesting that SPVD development was relatively delayed in these clones. The SPVD damage scores for 7-C127-Y10 and NASPOT 11 also indicated relatively delayed symptom severing. Most of the clones had maximum SPVD severity scores on scoring date 4 and thereafter had slight drops in scores suggesting some form of recovery. Overall, the event 7-C127-Y10 was most promising; its performance compared well with the resistant check.

**Introduction**
Sweetpotato Virus disease (SPVD) is the most economically important disease of the crop in Uganda; Incidence of SPVD ranges from Low (0-20%) in northern Uganda to high (50-95%) in Central and southern Uganda; the disease can cause yield losses of up to 90%.. Both incidence and severity are closely associated with prevalence of vectors. Although a lot of advances have been made in breeding sweetpotato for resistance to SPVD, the disease still remains a problem especially in orange-fleshed varieties.SPVD is caused by dual infection of mainly sweetpotatochlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV). On its own, SPFMV causes mild or no symptoms. However, natural resistance to SPFMV and other viruses is broken upon co-infection with SPCSV. Unfortunately, limited resistance to SPCSV is known in sweetpotato. This research targeted both SPCSV and SPFMV using RNA silencing conferred by small interfering RNA (siRNA). Three hairpin constructs, pC127, pC227, and pCIP41 were used to generate transgenic events at the Donald Danforth Plant Science Center (DDPSC), USA and at the International Potato center (CIP). The construct pC127 was targeted at *RNAse III* gene from SPCSV-EA and *NIa* and *NIb* gene from SPFMV-EA, while pC227 was targeted at the 3' UTR of SPCSV-EA and SPFMV-EA and pCIP41targeted the *RdRp* gene of SPCSV-Ug and *N1b* gene of SPFMV-Nam1.On challenging the events with either SPCSV or SPFMV at DDPSC and CIP in screen houses, virus specific siRNAs were produced in most events while double infection with SPFMV and SPCSV resulted in some symptomless lines which showed undetectable virus titers upon dot blot hybridization for SPFMV.

The promising events required to be tested for resistance under natural field conditions of SPVD to confirm the above results. Against this background this research was conducted with the main objective of testing whether RNA-silencing mediated resistance to SPCSV and/or SPFMV is able to reduce spread of SPVD in the field. A total of 21 events imported from CIP and DDPSC (following recommended guidelines for handling GMOs) and 3 controls Huachano (susceptible), Ejumula (susceptible) and NASPOT 11(resistant) were planted in a Confined field trial (CFT) at Namulonge in a complete randomized block design with 3 replications. Each plot had 20 plants on two ridges of ten plants each; spacing was 1m between rows and 0.3m within the row. Each plot was surrounded by virus infected spreader rows of Ejumula to enhance the virus inoculum in the field. Two subsequent crops; second planted with "clean" cuttings from the first crop. Prior to planting the trial sweetpotato fields within the vicinity (100m) were destroyed with an herbicide. All procedures were in accordance to the Guidelines for Confined Field Trials and all other applicable confinement measures and documentation requirements. Data on SPVD incidence and severity recorded. Incidence was captured as number of diseased plants/plot while SPVD severity was scored on a scale of 1-9 where 1=no visible symptoms, and 9=very severe symptoms. Data was recorded every two weeks starting with 1 month after planting (MAP) to 1 month before harvest. Flower buds were removed from the plants every week.

**Results and discussion**

During the first planting, the whitefly population was low and hence SPVD symptom development was slow until scoring date 5. By the last date of scoring, the resistant check, NASPOT 11 had an incidence of 8.9% while the susceptible check Ejumula had an incidence of 50.9 and the wild check Hachauno had the highest incidence of 100%. During the second season, at scoring date 1, clones 4-C127-27, 9-C127-Y21, Ejumula (susceptible check), NASPOT 11 (resistant check), 7-C127-Y10 had SPVD incidences below 40%. However, by scoring date 5 only 7-C127-Y10 and NASPOT 11 had SPVD incidences below 60% suggesting that SPVD development was delayed in these clones compared to the others. The SPVD damage scores for 7-C127-Y10 and NASPOT 11 also followed the same trend i.e delayed symptom severing compared to the susceptible check. Most of the clones had maximum SPVD severity scores on scoring date 4 and thereafter there were slight drops in their scores tending to suggest some form of recovery. Overall, the event 7-C127-Y10 was most promising as its performance compared well with the resistant check, NASPOT 11.

**Acknowledgements**

The authors would like to express gratitude to the Howard Buffet foundation for funding this research

## 4.16 Panel discussions on medical biotechnologies

*Dr. Hannah Kibuuka*
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The Makerere University Walter Reed Project (MUWRP) is a non-profit HIV research program that was established in 2002 as a result of a memorandum of understanding between Makerere University and The Henry M. Jackson foundation for Advancement of Military Medicine Inc. of USA (HJF). The primary purpose of MUWRP is to develop, evaluate and provide interventions to mitigate disease threats of public health importance to Uganda. Activities include; vaccine development and building of vaccine testing capability in Uganda, conducting of epidemiological and basic research and provision of HIV Treatment, care and support and

surveillance of influenza and other emerging infections. Vaccines are developed using Recombinant DNA Technology and therefore research participants cannot get infections from the vaccines. The vectors so far used are replication deficient and vaccines tested so far have been safe.

MUWRP Preventive HIV vaccine trials in Uganda started in 2004 with a single site RV156 HIV vaccine trial that tested a four-plasmid DNA vaccine. This was followed by a Multi-site study which enrolled 324 participants from Uganda, Kenya and Tanzania testing 2 vaccines, a six plasmid DNA vaccine and rAd5 vaccine( 2005). Participants were healthy HIV uninfected participants. They were followed up for 24 months following last vaccination. Another trial tested 2 vaccines, Pennvax-G given by Biojector 200 or Cellectra boosted by MVA-CMDR (2011). This study collected blood and mucosal samples to evaluate for systemic and mucosal immune responses following vaccination. Currently, MUWRP is part of a multisite trial testing Ad26 and MVA mosaic vaccines with funding from Crucell.

MUWRP Ebola vaccine trials in Uganda started in 2009 with a study RV 247 where two vaccines were given to healthy adults; an Ebola DNA vaccine and a Marburg DNA vaccine given individually and concomitantly in 108 participants. Vaccines were provided by the Vaccine Research Centre (VRC), NIAID and NIH. The current Ebola vaccine trials are RV 422 evaluating safety and Immunogenicity of two vaccines: cAd3-EBO (Zaire) and cAd3-EBO (Zaire and Sudan) with an amendment to boost with MVA; and the multisite Ebola phase 2 trial that is evaluating Ad26 and MVA vaccines in Healthy adults, HIV infected adults and children aged 1-17 years who are enrolled in a stepwise fashion.

Potential biosafety risks from Recombinant DNA vaccines include: Risk of Investigational product (IP) exposure to dispensing staff and accidental dissemination into the environment, potential integration of vaccine into patient's chromosomes leading to insertional mutagenesis, and anti-DNA antibody formation with possibility of autoimmune diseases. Potential risks are mitigated by review and approval of protocols, research processes and other relevant documents by research regulators and a Biosafety committee and follow up of research participants to monitor for potential risks. In addition, appropriate hygiene; decontamination and waste management measures and dispensing safety measures must be in place to ensure a safe environment despite working with these recombinant products.

In conclusion, participation of East Africans in HIV and Ebola Vaccine research is critical in understanding immune responses to tested interventions in the African population and past studies show that the population is willing to participate in the research. Measures are in place to ensure a safe working environment with these vaccine products.

# Appendices

## Appendix 1: Program for the National Biosafety Forum 2016

| | Time | Event | Responsible person |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Day one 1st Feb 2016** | | | |
| | 12:00 | Arrival and Registration | Beth/Eliza |
| | | **Lunch** | Beth |
| | 12:30-2:00<br><br>2:00-2:20 | Welcome remarks:<br><br>Executive Secretary, UNCST<br><br>Chairperson, NBC<br>Chairperson, UNCST | Julius Ecuru |
| | | ***Session Chair: Prof John Opuda-Asibo*** | |
| | 2:20-2:50 | Keynote: Gene Technology: Past, Present & Future Trends | Jennifer Thomson |
| | 2:50-3:40 | Current state of play of international biosafety laws and their implications for biotechnology development in Uganda. | Harriet Ityang |
| | 3:40-4:00 | Challenges of Biosafety Regulation in Africa: ‘’Experience from ABNE” | Silas Obukosia |
| | 4:00-4:30 | **Tea/Coffee Break** | Ms. Beth |
| | 4:30-4:50 | Understanding key points of contention within the proposed national biotechnology and biosafety bill | Mr. Herbert Oloka |
| | | Communicating biotechnology more effectively | Barbara Zawedee |
| **Day 2- 2nd February 2016** | | | |
| **Session Chair: Dr. Charles Mugoya** | | | |
| | 9.00-9.30 | GM mosquitoes for malaria control | Jonathan Kayondo |
| | 9.30-10.00 | Insect resistant GM Maize | Michael Otim |
| | 10:00-10:30 | Herbicide tolerant GM Cotton | Thomas Areke |
| | 10:30-11.00 | **Tea/Coffee Break** | **Beth/Eliza** |

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|---|---|---|---|
| **Session Chair: Prof. John Enyaru** | | | |
| | 11:00-11:30 | GM banana resistant to nematodes | Wilberforce Tushemereirwe |
| | 11:30-12:00 | GM bananas resistant to banana bacterial wilt | Wilberforce Tushemereirwe |
| | 12:00-12:30 | GM bio fortified bananas (Vit A and Fe) | Jerome Kubiriba |
| | 12:30-1:00 | Drought tolerant GM Maize | Godfrey Asea |
| | **1:00 – 2:00** | **Lunch** | **Beth/Eliza** |
| **Session Chair: Prof. Phinehas Tukamuhaabwa** | | | |
| | 2:00-2:30 | GM cassava resistant to CMD | Titus Alicai |
| | 2:30-3:00 | GM Cassava resistant to CBSD | Titus Alicai |
| | 3:00-3:30 | GM Rice efficient in Nitrogen and Water use | Jimmy Lammo |
| | **3:30-4:00** | **Tea/Coffee Break** | **Beth/Eliza** |
| **Session Chair: Dr. Agaba Friday** | | | |
| | 4:00-4:30 | GM virus resistant Sweet potato | GorettieSsemakula |
| | 4:30-5:00 | GM disease resistant potato | Andrew Kiggundu |
| | 5:00-5:45 | Panel Discussion on Medical Biotechnologies | Dr Hannah Kibuuka, Dr Phillipa Musoke |

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