AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION
FONDATION AFRICAINE POUR LES TECHNOLOGIES AGRICOLES

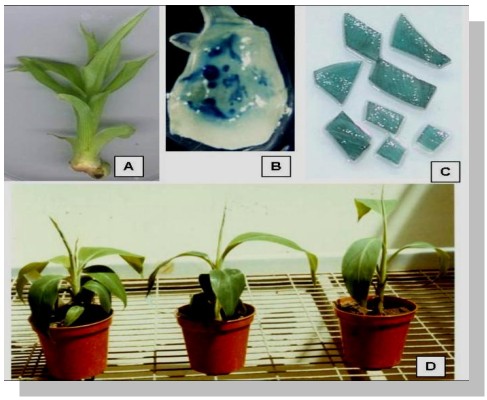
DEVELOPMENT OF TRANSGENIC BANANA (*Musa* spp) WITH RESISTANCE TO BACTERIAL WILT: A RISK ASSESSMENT APPRAISAL REPORT FOR SUB-SAHARAN AFRICA

PREPARED FOR

AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION (AATF)

BY

DR. N.O. AMUGUNE[1], PROF. J.I. KINYAMARIO[1] AND DR. J.W. KIMENJU[2]
[1]SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF NAIROBI
[2]DEPARTMENT OF CROP PROTECTION, UNIVERSITY OF NAIROBI



JULY, 2007

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACRONYMS

BTA	Biotechnology Trust Africa
GMO	Genetically Modified Organisms
GM	Genetically Modified
IITA	International Institute for Tropical Agriculture
KARI	Kenya Agricultural Research Institute
KEBS	Kenya Bureau of standards
INIBAP	International Network for the improvement of Banana and Plantain
KIPI	Kenya Industrial Property Institute
KIRDI	Kenya Industrial Research and Development Institute
NEMA	National Environmental Management Authority
ISAAA,	The International Service for the Acquisition of Agri-biotech Applications
JIC	John Innes Centre, UK
NARO	National Agricultural Research Organization, Uganda
NBC	National Biosafety Committee
GTIL	Genetic Technologies International Limited
OECD	Organisation for Economic Co-operation and Development, Paris

EXECUTIVE SUMMARY

Bananas (*Musa* spp.) are the developing world's fourth most important food crop after rice, wheat and maize in terms of gross value of production. The crop is grown in more than 100 countries throughout the tropics and sub-tropics, with an annual world production of about 98 million tonnes, of which one third is produced in Africa. Around 87% of all the bananas grown worldwide are produced by small-scale farmers for home consumption or for sale in local and regional markets.

Despite its importance, banana production is declining in sub-Saharan Africa mainly due to diseases and pests such as black Sigatoka (*Mycosphaerella fijiensis*), Fusarium wilt (*Fusarium oxysporum* f. sp. *Cubense*), bacterial wilt (*Xanthomonas campestris* pv *musacearum*), viruses (banana bunchy-top virus, banana streak virus), nematodes and weevils. The bacterial wilt previously restricted to Ethiopia is threatening banana farmers in the Great Lakes region. Declining yields are also due to decreased soil fertility due to shortened fallow periods.

Genetic modification to develop genetically resistant banana and plantain varieties are the basis upon which sustainable improved production for this crop can be developed. Although sources of resistance to many of the major pests and diseases are known in both landraces and wild species, development of disease-resistant banana by conventional breeding remains a difficult endeavour because of the long generation times, various levels of ploidy, sterility of most edible cultivars, and limited genetic variability (Tripathi *et al*., 2005). Furthermore, there are certain diseases for which sources of resistance are not known, an important example being banana bunchy top virus (BBTV) and banana bacterial wilt.

Biotechnology is now increasingly being used worldwide to facilitate and enhance the handling and improvement of bananas and plantains and to complement the efforts achieved through conventional breeding. Tissue culture has been used to produce disease free planting material thereby minimising disease spread through conventional suckers used in propagation. Genetic transformation is another approach geared towards development of disease and pest resistant bananas. Current efforts to create the resistance through genetic engineering are based on the premise that no known sources of bacterial wilt resistance exist in banana germplasm making improvement through classical breeding difficult.

In response to this challenge, the AATF and its partners are forging a common front to formulate a project which aims to transform banana against bacterial wilt (*Xanthomonas campestris pv. musacearum*), and make available resistant varieties for use by smallholder farmers in Africa. The transformation will involve the use of a ferredoxin-like amphipathic protein (*pflp*) which has been shown to be resistant to *Xanthomonas spp*. Our team was invited to prepare a risk assessment report that can guide the development and deployment of transgenic banana for sub-Saharan Africa.

This report documents the taxonomy, agronomy and importance of banana as a crop in sub-Saharan Africa. In addition, a range of constraints to banana production are reviewed with special attention to banana bacterial wilt and options for mitigating this disease through genetic transformation technology. Finally the report examines potential benefits expected to be derived from transgenic banana as well as potential environmental and public health concerns to be borne in mind when developing and deploying transgenic banana in sub-Saharan Africa.

1.0 BACKGROUND

Bananas and plantains (*Musa* sp.) are a major staple food, supplying up to 25% of the carbohydrates for approximately 70 million people in Africa's humid forest and mid-altitude regions. In terms of gross value of production, they are the developing world's fourth most important global food crop after rice, wheat and maize. World *Musa* production is currently about 104 million tonnes annually, of which bananas cultivated for the export trade accounts for only 10% (Tripathi, 2005). Hence, bananas are important for food security in the humid tropics where they form an integral component of the farming systems and provide income to the farmers.

Bananas are predominantly smallholder crops in sub-Saharan Africa, with growers being unable to afford costly chemicals to control pests and diseases which have significantly affected banana cultivation. For example, black Sigatoka (*Mycosphaerella fijiensis*), Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), bacterial wilt (*Xanthomonas campestris pv. musacearum*), viruses (banana bunchy-top virus, banana streak virus), nematodes and weevils cause significant crop losses worldwide. Banana bacterial wilt (BBW) also known as banana Xanthomonas wilt (*BXW*) is caused by the bacterium *Xanthomonas campestris* pv. *musacearum* and affects all banana types.

Xanthomonas campestris (Xcm) infection can result in heavy banana crop production losses and affect banana productivity by not only causing wilting and death of young banana propagules, but also by severe crop yield reductions in mother crop and subsequent ratoon plant production cycles. Xcm is gradually spreading in East Africa and if unchecked could result in massive losses. Studies in Uganda show that the disease attacks all varieties of banana, locally known as matooke. The disease has also been reported in the Democratic Republic of Congo, Rwanda and more recently has been identified in Tanzania and Kenya (Tripathi, 2005)..

There is a growing demand for improved varieties especially those that confer resistance to banana *Xanthomonas* wilt as bacterial diseases are difficult to control. The use of chemicals to control diseases is causing a lot of anxiety to the environmentally sensitive consumers. Breeding of resistant varieties is usually the best and most cost-effective method of managing bacterial diseases. However, attempts to develop bacterial disease-resistant varieties through conventional breeding have resulted in only limited success, as little existing genetic diversity shows resistance to BBW and because new races of the pathogen continue to appear (Tripathi, 2005). Even if resistant germplasm sources are identified, a conventional breeding cycle for improved banana germplasm development may be expected to take 6-20 years. Therefore, use of genetic transformation technologies, may provide an alternative approach to obtaining disease and pest resistant banana and plantain varieties (Tripathi, 2005).

Tissue culture (TC) used for germplasm exchange and rapid multiplication of high yielding and fast maturing varieties is now playing a critical role in banana production. For instance the tissue culture technology has been used to produce disease free planting materials and for rapid multiplication of high yielding varieties. However, though the TC bananas are initially disease free at the time of planting, they are susceptible to disease and pest attack once in the field. Therefore, efforts are being made to improve banana through transformation as improvement through conventional breeding is difficult due to sterility of the edible cultivars and lack of resistant germplasm especially to bacterial wilt.

Banana can be transformed by microprojectile bombardment of embryogenic cell suspensions or by use of *Agrobacterium.* The *Agrobacterium* system either uses embryogenic cell suspensions or apical shoot meristems. The use of transformation techniques may allow the production of disease resistant banana plants, in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced at the same time. In transgenic

banana, the introduced resistance should target nematode, fungus, bacteria and viruses. It may also be possible to incorporate other characteristics such as drought tolerance, thus extending the geographic spread of banana and plantain production, and thereby contributing significantly to the realization of food security and poverty alleviation in Africa (Tripathi, 2005). Transformation of sterile triploid dessert banana cultivars could certainly have a significant commercial impact.

Currently, no genetically transformed bananas are commercially available; however there is enormous potential for genetic manipulation of banana species for disease and pest resistance using the existing transformation systems. The transgenic bananas that have been developed so far are still under green house or undergoing field trials and are therefore not commercially available at present. Some of the transgenic banana lines under greenhouse or field evaluation include those that confer resistance to nematodes (Vain, 2006) , black and yellow Sigatoka (Banti-Kurti *et al*., 2001) as well as those expressing hepatitis B surface antigen (Kumar *et al*., 2005). However, there are no transgenic banana cultivars expressing resistance to bacterial wilt.

One strategy being considered to confer bacterial wilt resistance in banana is the use of a ferredoxin-like amphipathic protein (*plfp)* isolated from sweet pepper, *Capsicum annuum* (Lin *et al*., 1997). Pflp has been shown to delay the hypersensitive response induced by *Pseudomonas syringae* pv. *syringae* in non-host plants through the release of the proteinaceous elicitor, harpin Pss. The plants carrying the *pflp* gene showed enhanced resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) race 6 at various levels (Tang *et al.,* 2001). The *pflp* and *hrap*, would be good candidate genes for many susceptible crops to convert to resistant plant against many bacterial pathogens, such as, *Erwinia, Pseudomona, Ralstonia and Xanthomonas spp* through transgenic technology. The *pflp* gene has already been shown to be a useful candidate for genetic engineering strategies in rice to provide bacterial blight resistance as well as

transgenic orchids to confer resistance against *E. carotovora,* causing soft rot disease (Liau *et al.,* 2003).

Risk assessment is necessary before deployment of transgenic crops and transgenic banana will be no exception. There are two main concerns: environmental effects and human health. Functioning biosafety systems are required to ensure the safe handling and deployment of GM crops and equitable access to benefits by all sectors of communities. Along with the development of biosafety frameworks, there is a greater need to improve public understanding of biotechnology. The existence of functional biosafety framework is a means of ensuring safe testing and deployment of transgenic crops.

This document describes some of the issues that need to be considered before deployment of transgenic banana in sub-Saharan Africa. Because pests and diseases remain a challenge to banana production in Africa, AATF commissioned a study to examine the possibility of developing transgenic bananas that confer resistance to bacterial wilt, and the possible risks that may be associated with this. We undertook to do the task which involved desktop work such as literature review and internet searches as per the terms of reference outlined in 2.0 (Terms of Reference). We also carried out a field study based on a questionnaire that was filled by sampled farmers, traders and consumers in order to assess what the public feels about the introduction of genetically modified crops in particular the banana.

2.0 TERMS OF REFERENCE

The terms of reference for this risk assessment study were as follows:

1. Review and document the taxonomy, biology, agronomy, seed systems and importance of banana, including geographic distribution and history of use with special emphasis on utilization of the crop among smallholder farmers in Sub-Saharan Africa.

2. Review and document a range of constraints to banana production in Africa with special focus on threat posed by banana bacterial wilt, and existing coping strategies to this epidemic.
3. Describe a detailed account of various approaches to genetic transformation of banana particularly those targeted at the East African Highland Banana giving particular attention to efforts at transforming banana for resistance against banana bacterial wilt (focusing on genes of interest, description of vector DNA and transgene delivery process).
4. Evaluate environmental risks associated with transformation and eventual deployment of transgenic banana in the Great Lakes region of Africa.
5. Discuss potential human and animal health risks expected from consuming transformed banana and products thereof.
6. Further to the evaluation of risks, give an account of public perception and concerns regarding use of transgenic banana for food and feed in the region.
7. Prepare a Risk Assessment Appraisal Report to guide development, deployment and stewardship of transgenic banana in Sub-Saharan Africa, including a clear indication of possible decisions that should be considered by the project.
8. Submit the first draft of the Risk Assessment Report to AATF for review and comments.

Upon receipt of comments from AATF, prepare a Final version of the Risk Assessment Report for submission to AATF.

3.0 METHODOLOGIES AND WORK PLAN ADOPTED FOR THE STUDY

3.1 Methodologies

3.1.1 Introduction

Kenya and indeed countries in the Great Lakes region of Africa are signatories to the Convention on Biological Diversity (CBD), and are also parties to the Rio Declaration on Environment and Development popularly referred to as Agenda 21. Article 16 of Agenda 21 on technology transfer emphasizes that both access to and transfer of technology including biotechnology among contracting parties should make use of genetic resources in a way that should not cause significant damage to the environment. Article 19 of the CBD states the need for safe transfer, handling and use of any living modified organism resulting from biotechnology. The Cartagena Protocol on Biosafety articles 1, 15 and 16 emphasize the need for risk assessment and risk management to ensure adequate level of protection regarding the safe transfer, handling and use of genetically modified organisms resulting from the use of modern biotechnology that may have adverse effects on conservation and sustainable use of biological diversity, taking into account risks to human health, and specifically focussing on transboundary movement. This is in accordance with the precautionary approach contained in provisions of the CBD.

The rationale for undertaking this study is also in line with Kenya's National Environmental Management Authority (NEMA) requirements that Environmental Impact Assessment (EIA) and environmental audits be carried out on those specific activities that may impact on the environment as outlined in the Second Schedule (s.58 (1), (4)) of the Environmental Management and Co-ordination Act, 1999. These activities include: introductions of genetically modified organisms, agriculture, structures of a scale not keeping with their surroundings, storage dams, river diversions, mining, electrical generation stations, etc.

3.1.2 Outline of the Study

General Approach and Strategy

The consultancy services required were divided into the following parts/activities keeping in mind issues highlighted in the Terms of Reference.

Detailed Work Plan and Methodology

Task a: Mobilization

Prior to commencement of the work, one day of mobilization period from effective date of contract was needed during which the project team members settled their present assignments and made arrangements for transport, field and office equipment, etc.

During this mobilization period, the Team Leader prepared a detailed work programme. A so-called S-curve was prepared showing week by week, the expected percentage completion of the work, thereby clearly indicating the peak-load periods where the major production is expected.

The Consultant, during the mobilization period started collection of all relevant existing data for the planning of the surveys and studies needed, thus providing essential information for the refinement of the detailed work programmed.

Task b: Detailed work and report on the following:

The project team:

1. Reviewed and documented the taxonomy, biology, seed systems and importance of banana, including its geographic distribution and history of use with special emphasis on utilization of the crop among smallholder farmers in sub-Saharan Africa.
2. Reviewed and documented a range of constraints to banana production in sub-Saharan Africa with special focus on threat posed by banana bacterial wilt, and existing coping strategies to this epidemic.

3. Prepared a detailed account of various approaches to genetic transformation of banana, particularly those targeted at the East African Highland Banana, giving particular attention to efforts at transforming banana for resistance against banana bacterial wilt (focusing on genes of interest, description of vector DNA and transgene delivery process).
4. Evaluated environmental risks associated with transformation and eventual deployment of transgenic banana in the Great Lakes region of Africa.
5. Discussed potential human and animal health risks expected from consuming transformed banana and its products thereof.
6. Gave an account of public perception and concerns regarding risks and use of transgenic banana for food and feed in the region.
7. Prepared a Risk Assessment Appraisal Report to guide the development, deployment and stewardship of transgenic banana in Sub-Saharan Africa, including a clear indication of probable decisions that should be considered by the project.

Task c: Description of the Baseline Environment

The project team described the general baseline information on the environment where bananas are grown in the Great Lakes Region: including physical environment (topography, geology, climate and meteorology, etc.); biological environment (biodiversity, sensitive habitats, etc); and social and cultural environment (e.g. land use and cultural properties); and all other necessary environmental data.

Task d: Legislative and Regulatory Framework

The Consultant has:

1. Identified and described the pertinent regulations governing the agriculture, health and safety, land use control at the national levels in the Great Lakes Region of Africa in general and Kenya in particular.

2. Identified relevant ecological and socio-economic issues impacting on banana growing and use.

Task e: Occupation Health and Safety Concerns

The Consultant has analysed and described health and safety concerns on use of genetically modified organisms (GMOs) in particular transgenic banana.

Task f: Other relevant consultations

The Consultant has interviewed small holder banana growers, merchants, and agricultural officers on the possible growing and use of genetically modified crops like transgenic banana.

4.0 ORGANISATION OF THE REPORT OUTLINING THE VARIOUS PARTS

This report is organised into six sections as per the terms of reference already outlined in 2.0 above. Sections I-V were mainly desktop work involving literature searches in libraries and the internet. Section VI gives an account of the outcome of a public survey carried out to assess the feeling of the Kenyan public towards introduction of genetically modified crops in sub-Saharan Africa especially transgenic banana.

SECTION I

5.0 THE TAXONOMY, BIOLOGY, AGRONOMY AND SEED SYSTEMS: A REVIEW.

5.1 Importance and Geographic Distribution

Banana (*Musa* spp) is the fourth most important non-cereal starchy stable food crop in the world after cassava, sweet potato and yams (INIBAP, 1994). Among fruit crops, bananas and plantains are second only to citrus. They are regarded as the apples of the tropics and tend to be distributed within 30°N and 30°S latitude throughout the world, this is consistent with their centre of origin, the Southeast Asia region (Simmonds and Shephered, 1995).

Annual world banana production has risen from an estimated 89 million in 1998 (FAO, 1998) to 104 million tonnes in 2005 (Tripathi, 2005) of which only 10 -15% of this is exported. The implication is that banana is mostly a subsistence crop where it is grown. India and Latin American countries, particularly Brazil, are the largest world producers. Sub-Saharan Africa produces about 35% of the world's total with Uganda being the lead producer (Table 1).

Table 1: Production of bananas and Plantains in sub-Saharan Africa

Region	Production (1000 t yr^{-1})	Per Capita Consumption (kg)
West and Central Africa		
Angola	318	24
Cameroon	1274	85
Dem. Rep. of Congo	1831	69
Rep. of Congo	80	46
Cote d'Ivoire	1194	98
Gabon	159	142
Ghana	637	64
Guinea	318	46
Liberia	159	44
Nigeria	1990	25
East Africa		
Burundi	1506	88
Kenya	452	34
Madagascar	302	17
Malawi	151	9
Rwanda	2108	5
South Africa	151	180
Tanzania	1656	43
Uganda	8432	222
Others	301	
Total	**23 018**	

Source: FAO (1999)

Banana has a wide range of uses in different countries and regions in Africa. The most obvious uses include a variety of food products and beer while the crop remains are used as livestock feed or as mulching (Table 2). Banana is processed into different products such as flour, bread, cakes, jam juice, and

chips. Some banana varieties have specialized dietary uses like rejuvenating mothers after giving birth or as weaning food. The crop serves a unique role of food and animal feed security especially during extended drought periods. Although banana is largely grown as a food crop, it is an important cash crop in certain areas such as Central Kenya and South Western Uganda. In addition to sale of banana bunches other products like leaves, planting materials and fibres are considerable sources of income to farmers.

Apart from those universal uses, banana has many other uses that are of local importance. In Uganda, for instance, a bride returns to her maiden home after honeymoon and brings back banana bunches and a live chicken as an appreciation of her new husband. The male flower bud is used as a vegetable in Kenya and as a 'stopper' in many other parts of Africa. Bananas form part of the gifts that a bridegroom offers to the family of the bride during marriage negotiations. Moreover, banana beer is a common product in many parts of Africa. It is brewed for sale and it is drunk during cultural functions such as marriages and funerals. For example, a gourd of banana beer is offered to the man who inherits a widow after the burial of her husband in Uganda. In Western Kenya, a banana pseudostem is buried in place of the body in circumstances where a person is lost without trace. Banana fibres have numerous uses, including textile manufacture, for making ropes, string and thread, and for the production of various handicrafts. A broad range of handcrafts such as hats, lampshades, handbags, and tablemats can be made from banana fibres. Banana leaves are commonly used as umbrellas and in thatching houses because they are large and waterproof. Their large size also makes them useful as wrapping materials, making of beddings, bowl and table covers, to cover food while cooking, temporary mats, and plates. Banana sheaths are a common feature as cushioning to protect bunches in the process of handling and transportation on bicycles, handcarts, cars and trucks.

Almost all parts of the banana plant have medicinal effects. The flowers are used in treatment of bronchitis, dysentery, ulcers, and diabetes. The astringent plant sap is used to treat cases of hysteria, epilepsy, leprosy, fevers, haemorrhages, acute dysentery and diarrhoea. It is also applied on pimples, insect and other stings and bites. Young leaves are placed as poultices on burns and other skin afflictions. The roots are administered to treat digestive disorders, dysentery and other ailments. Antifungal and antibiotic principles have been reported in the peel and pulp of fully ripe bananas. It is alleged, in some areas, that smoking of dry banana peels, unlike tobacco, induces hallucinogenic effects and thus reduces depression.

Table 2: Major uses of banana in Africa			
Main Uses	Banana product	Main varieties	Country/Region

Cooking	Bunch	Nakabululu, Barabeshya, Kiganda, Uganda green, Mshale,	Continent-wide
Dessert	Bunch	Gros Michel, Cavendish,	Continent-wide
Roasting	Bunch	Gonja, Mshale, Mzuzu, Mkono wa Tembo	Rwanda, Burundi, Uganda, Tanzania
Alcoholic drinks	Bunch	Kayinja, Bluggoe, Mazizi, Isige, Nshembire	Rwanda, Burundi, Uganda, Tanzania
Livestock feed	Banana peels, pseudostems, corms, leaves	All varieties grown in Africa	Continent-wide
Processed poducts	Bunch	Cooking and dessert types	Great lakes region
Source of ash	Dry banana peels are burnt	Any variety	Western Kenya, Uganda
Venue making	Banana leaves	Any variety	Continent-wide
Wrapping material	Leaves and dry sheaths	Any variety but some varieties are more popular	Continent-wide
Source of fibre for making handcrafts	Dry leaves and sheaths	All varieties	Continent-wide
Cleaning of dead bodies and hands after burial	Pseudostem juice	Any variety	Uganda
Medicinal	Banana juice from corms used to treat pimples	Any variety	Uganda

5.2 TAXONOMY

Banana belongs to the order Zingiberales in the family Musaceae. The family contains two genera, *Musa* and *Ensete* (Simmonds, 1966; Cobley and Steele, 1976). Members of the *Ensete* are distributed in a wild state in Africa from the Cameroon throughout East Africa down to Transvaal in South Africa. A few species are also found from northeast India to the Philippines and Papua New Guinea (Purseglove, 1972). The genus *Ensete* differs from *Musa* by being monocarpic, non-suckering with a distinctively swollen base, and having large-sized seeds while *Musa* produces suckers and has small seeds (Cobley and Steele, 1976; Samson, 1992).

The genus *Musa* contains 30-40 species which have mainly resulted from mutations of the wild *M. acuminata* and the seedy *M. balbisiana* (Stover and Simmonds, 1987). Edible bananas and plantains are found in this genus. All the wild species in this genus are diploids (2n = 2x = 14, 18, 20, 22) and originated from South East Asia (Stover and Simmonds, 1987). The genus *Musa* is divided into five series, based mainly on the basic chromosome numbers, orientation and arrangement of flowers in the inflorescence (Argent, 1976; Simmonds and Weatherup, 1990a). Series *Musa* is the largest with 13-15 species, the most diversified and considered the oldest (Purseglove, 1972). It is widely distributed, extending from Southern India to Japan and Samoa (Purseglove, 1972). The series *Musa* has the basic chromosome number of eleven.

5.3 BIOLOGY

A banana plant is composed of the corm (rhizome or bulb), pseudostem, leaves, bunch and roots. The plant reproduces asexually from suckers that arise spontaneously from the corm. The primary banana plant and the budding suckers constitute the banana mat or stool.

The corm is the underground basal part of the plant. In longitudinal section, it is composed of the cortex, central cylinder and shoot buds. The central cylinder acts as a food reservoir for the growing plant. Banana roots are adventitious and

arise from the corm. They are extensive and found in the upper 60 cm of the soil. Depending upon the soil condition, they may grow in a lateral direction in excess of 1.5m. One corm may have 400-700 roots.

The upright portion of the plant (stalk) grows from the top of the corm. It supports the aerial pseudostem, which terminates in the bunch. The pseudostem is comprised of crescent (C-shaped) leaf sheaths. The tips of the leaf sheaths give rise to leaves. Leaves are comprised of the petiole, which is continuous with the midrib, and lamina of the leaf sheaths. Leaf production ceases after shooting. A normal plant maintains an average of 14 photosynthetically active leaves at any time before shooting. Throughout its life, a pseudostem produces an average of 65 leaves.

Inflorescence occurs when the aerial stem pushes through the pseudostem. Due to gravity, the inflorescence that begins in vertical growth later gets turned downward. The inflorescence is composed of proximal floral with developed ovaries, styles and stigmas that develop into the banana bunch. The male bud is formed from degenerated stamens and distal clusters. Each floral cluster is punctuated by a bract, which is shed as the bunch matures. As the male bud grows, mature flowers are shed, and at maturity, small male bud is apparent at the tail end of the floral axis. The process of inflorescence formation varies from one variety to another.

The bunch is formed at the apex of the aerial stem and consists of a hand of 10-16 fingers. The taste, size, colour and shape of the finger and as well as the shape of the bunch, are variety-specific. Edible bananas are seedless and parthenocarpic.

There are four phases of growth of a banana plant. In the first stage, growth is vegetative producing maximum number of leaves and takes 11 months for most cultivars. In stage two, inflorescence occurs, which takes one month followed by

bunch development that takes six months. Senescence takes two months to complete the cycle. During this final phase, the plant degenerates, the leaves and roots die, but the pseudostem remains to serve as a source of water and nutrition for the developing peeper.

Genome composition has played an important role in the classification of bananas. Majority of *Musa* spp are triploids from mutations of *M. acuminata* in tropical wet areas and *M. balbisiana* in the semi-arid tropics. With the basic number of n=11, the diploids (AA) are 2n=22, triploids (AAA) 3n=33 and tetraploids (AAAA) 4n=44, all from *M. acuminata.* When these mutate, they form AAB, ABB etc which are plantain varieties. New materials arise from spotting e.g. chimera. The major genomic groups therefore include diploids (AA, BB, AB) triploids (AAA, AAB, ABB) and tetraploids (AAAA, AAAB, AABB, ABBB). ABB is very rare; it has small fingers hence not good for marketing but is more resistant to Panama disease. The tetraploids: AABB, AAAA, AAAB and ABBBB are very rare but exist in the wild.

Genomic groups are composed of clones on the basis of morphological characters. Clones that share similar characteristics are considered to have arisen from a single base clone by mutation to form subgroups. Jones (2000) and Robinson (1996]) provide a list of the subgroups and some important clones that constitute each genomic group in *Musa*. It is worth noting that the bananas that constitute each genomic group can be very different. For example, the AAA group contains the sweet dessert bananas as well as the cooking bananas of the East African highlands. Similarly, the AAB group contains the plantains that are cooked before eating and the Pome subgroup that is used as dessert bananas in some countries. The ABB group appears to be rather homogeneous because all clones are used entirely for cooking.

Banana cultivars with the S and T genomes have been identified in Papua New Guinea (Shepherd and Ferreira, 1984; Arnaud, and Horr, 1997). Genomic groups

with the S genome include AS, AAS and ABBS, while those with the T genome are AAT, AAAT and ABBT.

5.4. Agronomy

5.4.1 Propagation

(i) Suckers and corms

Suckers are emerging plantlets while corms are part of the mother rhizome with lateral buds for regeneration. Planting materials can be obtained from established mats (commercial plantation), multiplication plots (banana nursery) and tissue culture laboratories.

Suckers are of different types namely peepers, sword, water and maiden. The best is sword, which is 1-1.5m tall (Mbwana *et al*., 1998). The corm is excavated and cut into longitudinal slices each of which should bear one or more eyes. The corms and suckers can be dipped in hot water at 55^{o}C for 20 *minutes* to control nematodes and weevils. In the absence of suckers and corms bull heads are used, these are the harvested plants trimmed to a height 1.5m from the pared corm.

(ii) Tissue culture

These are plants that are produced from the growing point of a plant under sterile conditions in the laboratory. The use of tissue culture (TC) plants enables rapid and large-scale multiplication of bananas for instance 1000 plants from one plant. Advantages of TC are: they have a faster growth rate and tend to flower earlier (9 months) compared to conventional suckers (>15 months). An evaluation of micro- propagated East African banana (Musa AAA EA) in Tanzania found them to fast maturing (Msogoya *et al.*, 2006). They are disease free, grow faster and more vigorously and hence yield more.

The sterile operational nature of tissue culture procedures excludes fungal, bacteria, and pests from the production system, which means that Sigatoka,

Panama disease, weevils, and nematodes cannot be transmitted through the TC micro-propagation process. However, viruses, such as the banana bunch top and the episomal form of banana streak virus, are not eliminated by tissue culture sterilization methods unless measures are taken to prevent the transmissions from happening (e.g., virus indexing).

5.4.2 Site Selection

The site should be sheltered from prevailing winds. Hail belts or storm areas as well as those areas prone to water logging and salinity should be avoided. The area should be close to water supply for irrigation where necessary.

5.4.3 Land preparation

Ploughing should be deep (200-300 mm) followed by harrowing to produce a fine tilth, which reduces compaction. Soil conservation measures should be undertaken on sloping land. Bench terraces should be constructed on slopes more than 25% to cater for single or double rows of bananas. On gentle slopes contour channel banks can be made and banks covered with a binding grass.

Large planting holes are dug where deep ploughing is not possible. Planting holes should be at least 60cm x 60cm x 60cm or 60cm in diameter and depth. Bigger holes of 90cm x 90cm x 90cm are preferred in dry areas. For tissue culture plants, deeper holes are recommended because the rhizome is often pushed above the soil level early due to the rapid and early production of suckers. Top soil up to 30cm depth should be mixed with 40-60kg of well decomposed manure and 200g of DAP (Diammonium phosphate). Half of this mixture is then returned to the hole to a depth of 30cm after which the planting material is placed into the hole and the rest of the soil then follows. The sub-soil can be used to make a basin around the plant. Bananas may be grown perennially on one location as long as pests and diseases are controlled and fertilizers are applied annually.

Spacing depends on the cultivars and field management (i.e. irrigation practice). For tall cultivars the plants are spaced at 4.0m x 4.0m (625 plants/ha), medium cultivars at 3m x 3m (1100 plants/ha) and short ones at 2m x 3m (1667 plants/ha).

5.4.4 Planting

Planting should be at the onset of the long rains. Before planting, old roots are removed and suckers immersed in warm water (52^{o}C) for 20 minutes. Alternatively the suckers are treated with pesticides to control nematodes and borers. TC bananas do not need this treatment.

5.4.5 Weed Control

Bananas have shallow roots hence shallow weeding is recommended. Weeding is done more frequently at the early stages of crop establishment; afterwards the leaf canopy suppresses weed growth thus reducing the frequency of weeding.

Selected herbicides can be used but only when the plants are over 2m tall and the leaf canopy is raised. Paraquat is effective on broad-leaved weeds while glyphosphate (Round-up) can be used to control the grasses.

5.4.6 Fertilizers

Fertilizer should be applied twice a year during the rainy seasons. Manure at the rate of 40kg per stool mixed with 200g of CAN is recommended on a yearly basis.

5.4.7 Sucker management

This is achieved by desuckering or thinning. Bananas produce a lot of unnecessary suckers, which deplete the stool of nutrients and provide unnecessary shade. Thinning should be done every four to six weeks to leave 3 plants at different stages. The stages are: bearing or nearly bearing mother plant,

large daughter sucker (maiden) and a small grand daughter ("peeper"). When the other suckers are culled, the middle is gorged out to kill the growing point.

5.4.8 Mulching

Mulching conserves moisture, suppresses weeds, controls soil erosion reduces soil compaction and increases soil fertility. Mulches used include dead organic mulch (banana leaf and pseudostem, grass, coffee husks) and living mulch which is a legume cover crop intercropped with bananas (e.g. groundnuts and beans). The mulch should be evenly spread and kept away from the base of the plants.

5.4.9 Leaf removal (deleafing)

Dead or dried leaves that hang down the pseudostem should be removed and used as mulch. Green functional leaves should never be removed as this reduces bunch weight. Leaves scaring or rubbing the fingers are removed to improve fruit quality.

5.4.10 Propping (forking)

Lodging in banana is common. It mainly due to weak anchorage, damage by weevils and nematodes, poor plant material, over crowding, or production of extremely large bunches, and strong winds. The plants should be propped up with wooded poles (double poles wedged or forked against the throat of the plant under the peduncle; however, care should be taken to avoid bruising the bunch. Bunches can also be supported by tying a rope ("manikka") to adjacent plants with bunches leaning in opposite directions. After a bunch is harvested, the pseudostem is removed.

5.4.11 Bunch trimming

The male flower bud is usually broken off when the bunch has fully emerged because it competes for food and is a shelter for thrips and mites. The male flowers should be removed 8 to 12 days after the bunch emerges so as to reduce thrip infestation and disease (cigar-end rot).

5.4.12 Harvesting

Bananas are usually harvested when bunches are about 75% mature. At that stage, the angles on the fingers become less prominent, upper hands change colour to light green, and the floral remains can be easily rubbed off the tips. Generally, this stage is reached 75 to 80 days after the opening of the first hand. With tall cultivars, the pseudostem must be slashed partway through to facilitate bending and harvesters pull on the leaves to bring the bunch within reach. Harvesters usually work in pairs to hold and remove the bunch without damaging it. A sizeable portion of the stalk (15–18cm) must be left attached to the bunch to serve as a handle during subsequent handling and carrying

5.5 Seed Systems

In Africa, availability of seed has often affected farmers' ability to sow a crop (Cromwell, 1996), yet seed is recognized as the factor, which sets the upper limits on production (Srivastava and Jaffee, 1993; Morris, 1998). Majority of Africa's smallholder farmers are poor, more so in sub-Saharan Africa and therefore cannot afford quality seeds or planting material at rates that appeal to suppliers, this leads to market failure (De Vries and Toenniessen, 2001). Like the general situation for most crops, banana seed system in sub-Saharan Africa is basically underdeveloped. Banana is propagated by means of suckers rather than true seed but the acquisition and dissemination of superior ones whether local or improved is still at nascent stage.

Farmers saved suckers are the main source of planting material (De Vries and Toenniessen, 2001). Although several reports suggest that farmers make their selections on the basis of quality characters in the field (Wright *et al.,* 1995), studies in Ghana and Zambia show less than 25% of farmers actually select seed in the field (Walker and Tripp, 1997). A recent survey conducted by IITA in Cameroon showed that 89.3% of farmers procured their planting materials from earlier crops (Hausa *et al*., 1998).

Dissemination of superior suckers in Africa has in recent times been seriously constrained by quarantine regulations due to banana streak virus in tissue-cultured germplasm (Vuylsteke *et al*., 1998). The bulkiness of banana raises the cost considerably in terms of labour and transport, which deters many farmers from acquiring good suckers unless the advantages are obvious. Bulkiness is a big impediment to take-off of small-scale banana enterprises.

The work on tissue cultured banana in Kenya was begun about a decade ago at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The first initiative was supported by ISAAA (The International Service for the Acquisition of Agri-biotech Applications) and KABP (Kenya Agricultural Biotechnology Programme) of BTA (Biotechnology Trust Africa) who funded the banana TC project implemented at JKUCAT and KARI. This initiative involved selection of varieties, TC production, quality control and assurance and training in nursery management (BTA, 2002). In addition, production and sale of TC banana has been taken up by a few private enterprises such as Genetic Technologies International Limited (GTIL) especially in Central province of Kenya where they are gaining popularity.

Dissemination of improved banana requires government intervention. In Kenya, for instance, the Ministry of Agriculture, in conjunction with research institutions, has embarked on ill-funded distribution of tissue-cultured banana in the main growing areas. Bulkiness of tissue culture plantlets, coupled with inaccessibility of the outlets dealing in TC is a major impediment to distribution of the materials. At the National Agricultural Research Organisation (NARO), Uganda the short term approach to banana improvement includes assembling of local and foreign banana germplasm for evaluation and selection of resistant or tolerant cultivars, propagation of superior, clean planting materials through tissue culture, and importation of hybrids from other breeding centers such as IITA for evaluation against local pests and diseases. The long-term strategy includes breeding for resistance with genetic transformation (Kikulwe *et al*., 2005).

SECTION II

6.0 CONSTRAINTS TO BANANA PRODUCTION

Banana productivity is declining against an increasing demand resulting from an increasing number of consumers. The decline in banana yield is mainly attributed to damage by pests and diseases and diminishing soil fertility, coupled with poor crop husbandry (Karamura, 1998; Seshu-Reddy and Lubega,1993).

Various reports have cited soil degradation in the form of soil erosion and loss of soil fertility as one of the major causes of banana yield decline (Bekunda and Woomer, 1996; Woomer *et al*., 1998; Sseguya *et al*., 1999; Achan, 2001).

There is plenty of internal banana trade in sub -Saharan Africa but little export trade has developed. This is attributed to lengthy distances to seaports and high perishability of bananas. Low value to volume ratio makes it uneconomical to export banana because of the expensive air and freight space when compared to ornamentals which are the choice crops for export. Pests and diseases are however the main drawbacks to banana growing.

6.1 Major pests and their management

6.1.1 Banana weevil (*Cosmopolites sordidus*)

Banana weevil is ranked among the most important insect pest of bananas in many parts of sub-Saharan Africa. Adult weevils are initially brown but later turn black (Plate 1). They feed mainly on the rotting plant debris. The larvae are white with brown heads and feed their way into the corms and pseudostems. Symptoms are indicated by rough circular tunnels in the corm and pseudostem that increase in size with the growth of the larvae (Plate 2). Plants are stunted and have weak stems and yellow leaves. Eventually a rhizome with numerous tunnels is reduced to a blackened mass of rotten tissue. The pseudostem snaps

at the ground level (Plate 3). Controls include Integrated Pest Management (IPM) including addition of manure, field sanitation, clean planting material, trapping, and use of neem extracts. Natural enemies like *Plassius javanus* and entomopathogenic nematodes have been used successfully in controlling the weevil. Another control option involves planting varieties resistant to banana weevil.

Plate 1: Banana weevil (adult stage)

Plate 2: Damage to the corm by banana weevil

Plate 3: Snapping of bananas due to damage by weevils

6.1.2 Banana thrips (*Hercinothrips bicintus*)

Thrips are insects that attack young fruits from which they suck up plant sap. The infested fruits have silvery or brown patches. Skins of severely infested fruits may crack. Control measures include field sanitation (detrashing), cutting off of dry leaves, regular desuckering (thinning) to avoid dense canopies which provide a microclimate that is favourable to the thrips. Bagging of bunches after complete emergence using polythene sleeves and spraying infested bunches with Diazinon, Fenithion or Fenitrothion.

6.1.3 Banana aphids *(Pentalonia nigronervosa)*

Apids are soft-bodied insects, which are usually found on the underside of the plant leaves. The aphids are vectors of viruses, which cause bunchy top disease. Direct damage resulting from sucking of plant sap is negligible but they are important as vectors of diseases. Banana aphids appear as clusters of brown, shiny, soft-bodied insects. The aphids occur in higher densities under older leaves particularly those that are near the base of the stem and are often attended by ants. Control measures include de-leafing and spraying the stems of young seedlings using Parathion. Virus infected plants should be dug out and destroyed to reduce spread of viruses; and lastly planting materials should be obtained from clean plantations.

6.1.4 Banana nematodes

Nematodes are a major limiting factor to banana production with the lesion *(Pratylenchus goodeyi, P.coffeae*), burrowing *(Radopholus similis*), spiral (*Helicotylenchus multicintus)* and root knot (*Meloidogyne incognita*) being the most common species in Africa. The severity of damage caused by nematodes ranges from slight to severe (which is characterized by toppling) depending mainly on the mode of parasitism of the different species, soil type, climate and susceptibility of the cultivars grown. The nematode destroys the root and rhizome tissues, resulting in poor anchorage of the plant and thus a tendency to uproot or topple. Losses are especially high during strong winds and heavy rains. Other

losses attributed to damage by nematodes include: stunted growth, longer period to maturity, small bunches (reduced bunch weight) and reduced longevity of banana plantations.

At least four root knot (*Meloidogyne* spp.) nematode species are frequently found in association with the banana: *M. incognita, M. arenaria, M. javanica* and *M. hapla.* The most characteristic symptom of root-knot nematode damage is galling (knots) on primary and secondary roots. Many other nematode species have been detected in banana roots and rhizosphere. They include *Rotylenchus reniformis, Hoplolaimus pararobustus, Helicotylechus mucronatus, H. macrophalus* and *Cephalenus emagitus.*

Nematodes are usually not evenly distributed in fields. The first indicator of nematode infection is the appearance of patches of poorly growing plants despite favourable conditions for crop growth. General symptoms of nematode damage include yellowing of leaves, stunted growth and excessive wilting during hot or dry weather. Black or dark red lesions (wounds) on the surface of the roots that extend to the cortex appear. Uprooting or toppling over of plants especially those bearing fruits. Root-rot, induced by fungal and bacterial organisms, is usually associated with plant tissue that is damaged by nematodes.

Control measures include use of nematode free planting materials, for example, T.C. banana seedlings. Roots of suckers from own farm should be trimmed before dipping in hot water at 55 $^{\circ}$C for 20 minutes. The suckers could also be dipped in nematicides. Regular spot application of granular or liquid nematicide formulations is also effective. Propping fruit stems reduces uprooting of plants. Crop longevity is extended if plants are regularly mulched with organic amendments (compost, green manure or animal manure). Reducing movement of infested soil from one point to another by use of soil conservation measures should be encouraged.

6.2 Major diseases and their management

6.2.1 Panama disease (*Fusarium* wilt)

Panama disease or fusarium wilt caused by the fungus *Fusarium oxysporum* f.sp. *cubense* attacks the pseudostem and corms of susceptible cultivars. The disease is known to be widespread in the main banana growing regions in Africa: East and West Africa (Ploetz, 1991). The fact that the pathogen remains in the soil for up to 30 years after the soil is infested makes it one of the most important banana diseases. The pathogen occurs in three races (Race 1, 2 and 4). Race 4 is one of the most dreaded because 'Cavendish' bananas which are resistant to other races succumb to it. The most susceptible cultivars are Gros Michel (Kampala), Apple banana (sukari ndizi), Muraru, Bluggoe (*Bokoboko*) and Psian Awak. Tolerant varieties are mainly in the Cavendish group.

Plate 4: Banana plants infected with *Fusarium* wilt

Yellowing of leaves begins along the margins and advances towards the midribs progressing from older to younger leaves as the entire plant dries up (Plate 4). Leaf petioles turn brown and bend or become twisted (buckle). Brown spots of various shapes and sizes appear on yellow leaves. Pseudostems frequently split longitudinally just above the soil level. The outer leaf sheaths may separate from the pseudostem and collapse. Diseased rhizomes and pseudostems release offensive smell due to rot caused by secondary pathogens. The vascular tissue is discoloured.

The main control measure entails total removal and "on the spot" (*in situ*) burning of infected plants. Cavendish varieties should be planted in infested soil because they are resistant to most of the pathogenic strains of the fungus. Other measures include reducing movement of infested soil from infection locus using soil conservation methods; planting disease free suckers especially tissue culture plantlets in disease free fields. Garden tools should be sterilized by flaming to reduce spread of the disease within and between fields.

6.2.2 Sigatoka

6.2.2.1 Black Sigatoka

This is a leaf spot disease caused by the fungus *Mycosphaerella fijiensis*. Black sigatoka (black leaf streak disease, BSLD) is one of the most important leaf spot diseases in the world. It is a major problem in Africa causing yield losses of up to 50%. The disease affects mainly the young leaves. Initial symptoms are tiny black streaks (1-2mm) on the under side of the leaf, which enlarge to 5-10mm with no distinct border. The streaks coalesce into black leaf spots that later merge to kill the entire leaf. Secondly banana bunches mature prematurely.

6.2.2.2 Yellow Sigatoka

This is also a leaf spot disease caused by *Mycosphaerella musicola.* It resembles black sigatoka but yellow borders surround the streaks. Initial symptoms are pale yellow streaks on the upper side of the leaf surface that enlarge to form dead areas with yellow halos and grey centres, otherwise all the other symptoms resemble those of black sigatoka. Control by use of clean planting material significantly reduces the spread. Routine detrashing (leaf removal) and burning of infested leaves and finally pruning to open up the canopy to allow more sunlight that discourages germination of the causal agent is a good control measure.

6.2.3 Cigar-end rots (*Verticillium theobromae*)

This disease is increasingly becoming an important constraint in many parts of Sub-Saharan Africa. The Dwarf Cavendish and Gros Michel varieties are particularly susceptible. Symptoms appear as rot on immature fingers with an ashy appearance (spores) on fruit tips and resembles the tip of smoked cigarette, hence the name. The rot affects a few centimetres of the tip and develops along with the fruit growth. The pulp develops as dry rot and becomes fibrous. Control is by removal of dried floral parts from the fruit tips 8-11 days after bunch emergence. Bananas should be covered with a polythene bag (6/100 thickness) before hands emerge. Pruning to open up the canopy and spraying with fungicides are also good control measures.

6.2.4 Bunchy top disease

The disease is caused by a virus which is disseminated by the banana aphid *(Pentalonia nigronervosa).* Long distance dispersal however is primarily through planting infected materials. With the exception of Burundi, the disease is not widespread in East Africa and efforts should therefore be made to exclude it from disease free areas. The first symptoms are dark green streaks in the petioles and leaf veins then leaves of young suckers become chlorotic and curled. Infected plants are stunted in growth. Control measure is mainly by making sure planting material is disease free, infected plants should be uprooted and burned. Spraying with insecticides is recommended at the early stages of seedling establishment.

6.2.5 Banana Bacterial Wilt (BBW) or Banana Xanthomonas Wilt (BXW)

The causal agent is the bacterium *Xanthomonas campetris* pv. m*usacearum.* This bacterium affects all banana types and can affect any stage of the plant thus posing a big threat to bananas in sub-Saharan Africa. This little known disease was reported only from Ethiopia where it affects ensete *(Ensete ventricosum)* and occasionally cultivated bananas (Yirgou and Bradbury, 1974).

The first symptoms include discoloration at the tip of the flower and withering of the flower bracts. Other symptoms include yellowing, wilting and premature ripening in young plants (Plate 5). When the banana is cut, a pink-purple coloration confirms presence of the disease. Even in some cases where these other symptoms fail to show, the coloration is always seen (Plates 6 and 7). The plant dies within a month from the first appearance of any of the symptoms (Tripathi *et al*., 2004). Care must be taken not to confuse it with *Fusarium* wilt.

Plate 5 (A, B): Banana plants showing symptoms of Banana *Xanthomonas* wilt infection

BBW is readily spread by contaminated cultivation and cutting tools, in infected seed pieces (which may appear outwardly normal) and probably by insects that spread the bacterium from diseased to healthy inflorescences. The disease spreads rapidly. Within the last four years this disease has moved from Ethiopia into Uganda, Congo, Rwanda and Tanzania (Karamura, 2006). Credible reports indicate that bacterial wilt is already causing damage to banana in districts of western Kenya that neighbour Uganda.

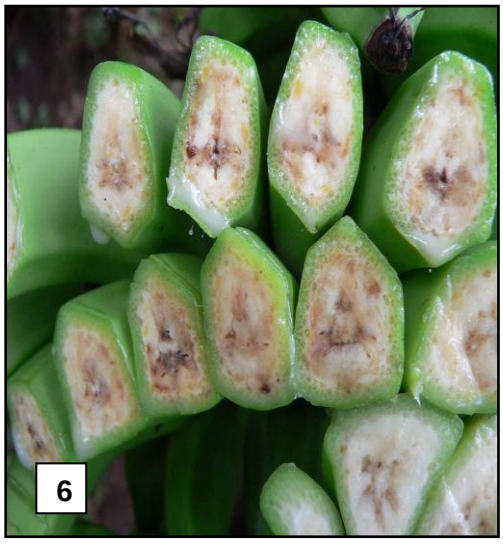


Plate 6: Tranverse section of infected banana fingers showing discolouration

Plate 7: Tranverse section of an infected banana pseudostem showing bacterial exudate

6.3. Management of Banana Xanthomonas Wilt

BXW is a classical example of a disease that can be managed using an integrated approach (Tushemereirwe *et al*., 2003). The Ugandan experience shows that the following components should form part of the integrated package.

- Timely removal of the male bud immediately the bunch is formed to reduce spread of the disease by insects that visit the flower to collect nectar.
- Use of disease-free planting materials.
- Destruction of infected plants by uprooting or injection with herbicides.
- Disinfect ion of tools like knives after using them to cut infected plants. Disinfection can be achieved by flaming or dipping the tools in chemicals such as sodium hypochlorite or alcohol.
- Banana residues collected from trading centers should not be used as mulch until they are fully decomposed.

- Farmers' own tools should be used in all farm operations. The practice whereby traders move from field to field buying and harvesting bananas with their own tools should be avoided.

SECTION III

7.0 GENETIC TRANSFORMATION OF *MUSA*

Genetic transformation has become an important tool for crop improvement. There are three main approaches that are generally used to insert DNA into a plant cell: vector-mediated transformation, particle-gun bombardment and direct DNA absorption. With vector-mediated transformation, a plant cell is infected with a bacterium or virus that, as part of the infection process, inserts the DNA. The most commonly used vector is the crown-gall soil bacterium, *Agrobacterium tumefaciens.* The particle-mediated transformation (particle bombardment), uses a tool referred to as a "gene gun," that transfers DNA into the cells by metal particles that have been accelerated, or "shot," into the cell. The particles are usually very fine gold pellets onto which the DNA has been stuck. With direct DNA absorption, a cell is immersed in the DNA, and an electric shock usually is applied ("electroporation") to the cell to stimulate DNA uptake.

Selectable markers, mainly antibiotic or herbicide resistance genes are then used to select transformed cells from the non-transformed ones. The neomycin phosphotransferase II (NPTII) and hygromycin (HGR) genes which confer resistance to the antibiotics kanamycin and hygromycin, respectively are the most commonly used. Herbicide resistance markers include those that confer resistance to phosphinothricin (PPT) and glufosate. Reporter genes such the *ß-glucuronidase* (GUS) gene are used to confirm transformation. The genes are driven by promoters such as the cauliflower mosaic virus 35S (CaMV35S) that allow expression in different plant tissues.

Relative success in genetic engineering of bananas and plantains has been achieved, enabling the transfer of foreign genes into the plant cells. Protocols for electroporation of protoplasts derived from embryogenic cell suspensions (Sagi *et al*., 1994), particle bombardment of embryogenic cells (Sagi *et al.,* 1995; Becker *et al*., 2000; Cote *et al*, 1997; Tripathi *et al*., 2005), and co-cultivation of

wounded meristems with *Agrobacterium* (May *et al*., 1995; Bosque-Pérez *et al*., 1998; Tripathi *et al*., 2003) are available for bananas and plantains.

7.1 Particle bombardment of embryogenic suspensions

The particle bombardment technology in banana combines particle bombardment transformation with a regeneration protocol using embryogenic suspension cultures derived from somatic cells. The technology is limited by the availability of cell cultures with a sufficiently high capacity for plant regeneration. An average of 2-3 transgenic plants per bombardment can be regenerated from a cell suspension with a good morphogenic potential. Transformed plants are ready to be established in the greenhouse six to eight months after bombardment. At present most of the transformation protocols use cell suspension, however establishing cell suspension is a lengthy process and cultivar-dependent.

7.2 Agrobacterium-mediated transformation

The *Agrobacterium* procedure offers several potential advantages over the use particle bombardment as it reduces the copy numbers of the transgene, potentially leading to fewer problems with transgene co-suppression and instability (Gheysen *et al*., 1998; Hansen and Wright, 1999; Shibata and Liu, 2000). The other advantage of the *Agrobacterium* system is the lack of requirement for high-tech equipment or sophistication in tissue culture and the assumption that only the desired DNA sequences are transferred to recipient cells. An *Agrobacterium*-mediated transformation system is also much faster when compared with particle bombardment of embryogenic cells.

The *Agrobacterium* technique is more widely applicable to a wide range of *Musa* cultivars irrespective of ploidy or genotype as it is based on the use of differentiated tissue that can be routinely regenerated into whole plants (Bosque-Pérez *et al*., 1998; Tripathi *et al*., 2003). The *Agrobacterium*-based banana transformation system as originally reported used explants containing the apical meristem or corm meristematic tissues which were wounded by micro particle bombardment with uncoated particles. After a brief recovery period, these

meristems were subsequently co-cultivated with *Agrobacterium tumefaciens* harbouring the plant transformation vector in the presence of acetosyringone, a known inducer of the *Agrobacterium* virulence genes. Antibiotic resistant plants were regenerated and DNA hybridization demonstrated that the transgenes were incorporated into high molecular weight genomic DNA and no residual *Agrobacterium* persisted in the plants. In addition, plantlets that had undergone multiple rounds of propagation maintained these genotypic and phenotypic traits.

Multiple disease resistance in banana is likely to be achieved by integrating several genes with different targets or modes of action into the plant genome. Technically, this can be done either in several consecutive steps or simultaneously. It has been shown that simultaneous gene transfer into banana can be performed by co-precipitation of a mixture of chimaeric gene constructs onto micro particles before bombardment. Transgenic plants regenerated after bombardment with such constructs contained unlinked genes at a frequency of 70 – 80%. As a result of these co-transformation experiments, transgenic banana plants containing up to six different genes have been obtained. These observations indicate that simultaneous bombardment of different plasmid molecules may be a convenient way for the introduction and perhaps co-expression of multiple genes in banana plants.

7.3 Current applications of genetic transformation in *Musa*

Most of the transgenic bananas produced so far harbour genes that confer resistance to diseases and pests. Such resistance genes known as R genes that mediate resistance to bacterial, fungal, viral, and nematode pathogens have been cloned from several plant species (Bent, 1996). Many of these *R* gene products share structural motifs, which indicate that disease resistance to diverse pathogens may operate through similar pathways.

7.3.1 Resistance to bacterial diseases

Several strategies have been employed to develop plants resistant to bacterial diseases. One such approach is by the use of antimicrobial peptides (AMPs) isolated from frogs, insects, and mammalian phagocytic vacuoles (Tossi *et al.,* 2000). Also being used are antibacterial lytic peptides, called cecropins, which are active against a wide range of plant pathogenic bacteria including *Erwinia* c*arotovora, E*. *amylovora, Pseudomonas syringae, Ralstonia solanacearum and Xanthomonas campestris* (Kaduno-Okuda *et al*., 1995; Nordeen *et al.,* 1992; Rajasekaran *et al*., 2001). Therefore, cecropins have been considered as potential candidates to protect plants against bacterial pathogens.

Attacins are another group of antibacterial proteins produced by *Hyalophora cecropia* pupae (Hultmark *et al*., 1983). Attacin expressed in transgenic potato enhanced its resistance to bacterial infection by *E*. *carotovora* subsp. *atrospetica* (Arce *et al.,* 1999). Another source of antibacterial proteins has been lysozyme, either from bacteriophage, hen eggs or bovine. There are evidences of efficacy of bovine lysozyme isozyme c2 (BVLZ) enzyme against a variety of *Xanthomonas campestris* strains, as a transgene, in both monocot and dicot crops such as tomato, tobacco, rice and potato (Mirkov and Fitzmaurice, 1995).

A ferredoxin-like amphipathic protein (*pflp*, formerly called AP1) isolated from sweet pepper, *Capsicum annuum,* is a novel plant protein that can intensify hairpin-mediated hypersensitive response (HR). This protein has dual function; an iron depletion antibiotic action and a hairpin triggered HR enhancement (Lin *et al*., 1997). The ferredoxin gene *pflp* acts together with a hypersensitivity response assisting protein (*hrap*) to confer hairpin-mediated response in plants. The *pflp* has been shown to delay the HR response induced by various pathogens like *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas* spp. in non-host plants through the release of the proteinaceous elicitor hairpin in various crops, including dicots like tobacco, potato, tomato, broccoli, orchids, and

monocots like rice (Lin *et al*., 1997; Huang *et al*., 2004). The patent holder for this gene is Academia Sinica.

Since the *pflp* and *hrap* genes have been shown to function in genetically engineered monocots such as rice with demonstrated efficacy against bacterial pathogens including *Xanthomonas* (Tang *et al*., 2001), their usefulness as transgenes for resistance to *Xcm* in banana has a high probability of success.

7.3.2 Development of EAHB varieties resistant to xanthomonas wilt

Several efforts have been made to transform the East African Highland Banana (EAHB) using standard vectors carrying the *ß-Glucuronidase* (GUS) intron gene in order to optimize the system. Tripathi *et al*. (2005) developed an efficient transformation system for EAHB cultivar Agbagba (AAB)] using *A. tumefaciens* harbouring the plasmid pCAMBIA 1201(Fig 1). This transformation was to augment and facilitate breeding of disease resistant banana and plantain for sub-Saharan Africa. Apical shoot tips were transformed using *Agrobacterium* strain EHA105 with the binary vector pCAMBIA 1201, having the hygromycin resistance gene as a selection marker and GUS-INT as a reporter gene. Transient expression of the *ß-glucuronidase* (*uid* A) gene was achieved in transformed apical shoot tips. The hygromycin resistant shoots were regenerated 4 to 5 weeks after co-cultivation of explants with *Agrobacterium*. The two step selection procedure allowed the regeneration of shoots which were uniformly transformed. The integration of the *uid* A gene was confirmed by polymerase chain reaction (PCR) and Southern blot analysis.

In this study by Tripathi *et al.* (2005), transformation based on regeneration from apical shoot tips was demonstrated. This process does not incorporate steps using disorganized cell cultures but uses micropropagation, which has the important advantage that it allows regeneration of homogeneous populations of plants in a short period of time. This study showed that *Musa* species cultivar Agbagba (AAB)] could be transformed and attempts should be made to

incorporate genes for disease and pest resistance, as well as abiotic factors (Tripathi *et al*., 2005).

The *uid* A gene driven by the CaMV35S promoter was used to confirm the transfer of transgene from super virulent strain EHA105 to an ordinary strain LBA4404 (Tripathi *et al*., 2005). Transient *uid* A gene expression, i.e. the development of blue loci after staining, was observed in 60 to 70% of the explants co-cultivated with EHA105 (1201) (Plates 8B, 8C). The explants co-cultivated with other strains showed expression of *uid* A gene in only 40 to 45% (C58 and LBA4404) or 20 to 25% (GV2260). The recovery of stable transformants mainly depends on the regenerative competence of the target tissues and their recovery after co-cultivation. The regeneration of explants after co-cultivation with various *Agrobacterium* strains was also compared. The explants co-cultivated with EHA105 regenerated at an efficiency of about 98% whereas with other strains the efficiency was 70% (LBA4404 and C58) and 60% with GV2260. The comparison of *uid* A gene expression and evaluation of regeneration efficiency after co-cultivation by various strains indicated that EHA105 was the best strain for transformation of apical shoot tips of the plantain cultivar Agbagba. This system is now being considered for transformation of banana against bacterial wilt (Tripathi *et al*., 2005).

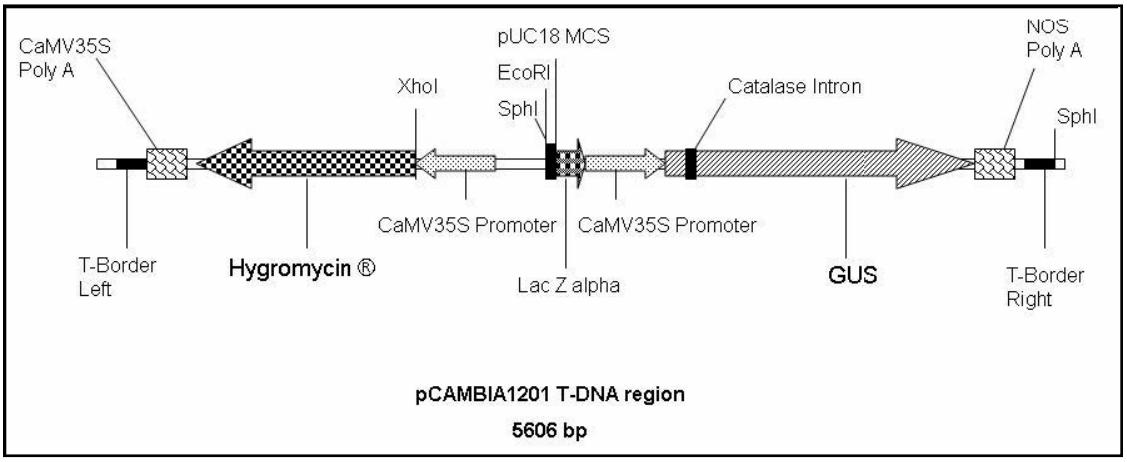


Fig.1: Schematic representation of T-DNA region of binary vector pCAMBIA 1201 (*Adapted from Tripathi et al., 2005***)**

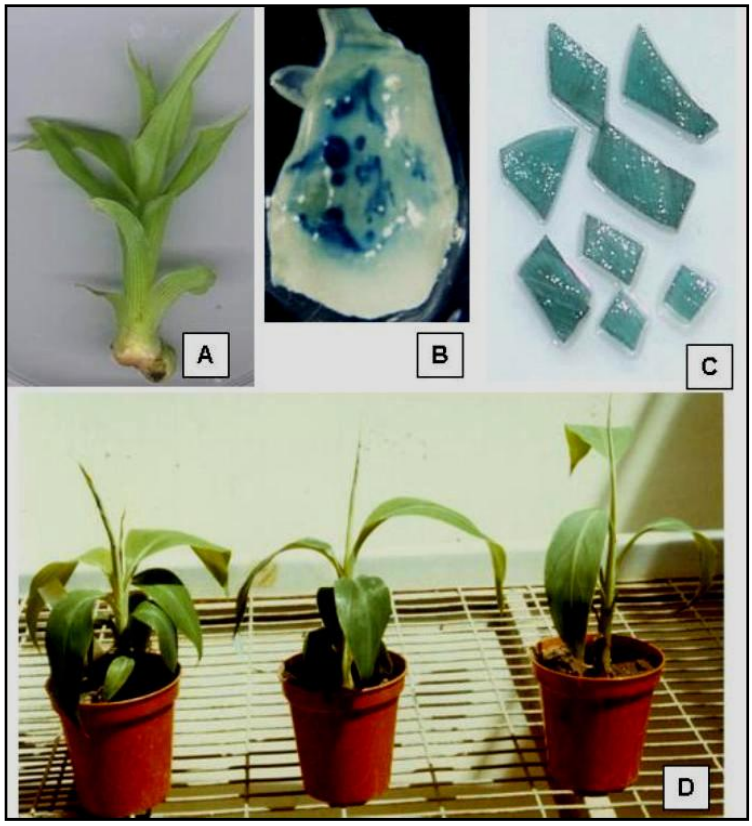


Plate 8: (A). Transgenic shoot regenerated on selection medium; (B). Transient expression of uid A gene, 48h after co-cultivation; (C). Stable expression of uid A gene in the leaves of the transgenic plants; (D). Transgenic plants in containment house (*Adapted from Tripathi et al., 2005***)**

The genetic transformation system developed and optimized at IITA is being considered for the introduction of bacterial wilt resistance into the EAHB banana, using *pflp* and *hrap* genes already demonstrated to confer resistance against bacterial wilt in other crops (Tripathi, 2005). The disease resistant farmer preferred banana varieties can contribute significantly to food security and poverty alleviation in the region.

7.3.3 Fungal disease resistance

As significant damage to banana production is caused by fungal pathogens, the introduction of genes conferring resistance to fungal pathogens is of prime importance. Genes coding for antifungal proteins that show broad antifungal activity *in vitro* have been introduced into a plantain landrace (Remy *et al*., 1998). Attempts have also been made to develop transgenic banana showing resistance to black Sigatoka and also modifying banana ripening characteristics.

A genetic transformation system has been developed for three *Mycosphaerella* pathogens of banana and plantain (*Musa* spp.) namely *Mycosphaerella fijiensis* and *Mycosphaerella musicola*, which cause black and yellow Sigatoka, respectively, and *Mycosphaerella eumusae*, which causes Septoria leaf spot (Balint-Kurti *et al*., 2001). The plants were transformed with a construct carrying a synthetic gene encoding green fluorescent protein (GFP). Most single-spored transformants that expressed GFP constitutively were mitotically stable in the absence of selection for hygromycin B resistance (Balint-Kurti *et al*., 2001).

Other efforts have focused on the use of antimicrobial proteins (AMPs) which are stable, cysteine-rich small peptides isolated from seeds of diverse plant species. These AMPs have a broad anti-fungal spectrum and show high *in vitro* activity against field isolates of *Mycosphaerella fijiensis* and *Fusarium oxysporum*, the two main fungal pathogens of *Musa*, while they exert no toxicity to human or plant cells. Five AMP genes have been introduced into embryogenic cell suspensions of banana and plantain using particle bombardment (Sagi *et al*., 1997). Large-scale molecular and biochemical characterization of the transgenic lines confirmed that a vast majority contained the foreign genes. Since black Sigatoka is the most threatening banana disease with yield losses of up to 50% worldwide, transgenic lines of EAHB expressing antifungal peptides are currently being screened for resistance against this fungus in greenhouse infection experiments. Applications for field testing in different countries are being

prepared or awaiting evaluation by the local biosafety committees (Tripathe *et al.,* 2004).

To study the expression of antifungal proteins in the transgenic plants, the AMP genes were placed under the control of various regulatory sequences (promoters) which had previously been shown to drive high gene expression in banana as well as in other monocots. Using specific antibodies, the concentration of two antifungal proteins in total leaf proteins was determined in transgenic plant extracts and results ranged from 0.05% to more than 1% depending on the promoter used. Addition of these extracts to germinating spores of a field isolate of *Mycosphaerella fijiensis*, the causal agent of black Sigatoka disease, resulted in a significant inhibition of fungal growth making these plants likely candidates to control this pathogen under field conditions. It has been shown that one AMP gene is also expressed in the fruit, opening the opportunity to create resistance against pre- and post-harvest diseases such as cigar-end rot and crown rot (Sagi *et al*., 1997).

Transformation of banana against Panama wilt using Human lysozyme (HL) gene has also been attempted (Xin-Wu Pei *et al*., 2005). Human lysozyme cleaves the ß-(1-4) glycosidic bond of peptidoglycan in the bacterial cell wall and of chitin in the fungal cell wall, thereby helping the host plant fight against the fungal and/or bacterial infection (Nakajima *et al.,* 1997, Miyuki and Kenji, 2000). The HL gene inhibits *Fusarium oxysporum* (FocR4) growth *in vitro*. To obtain transgenic bananas (*Musa* spp.) that are resistant to Panama wilt (*F. oxysporum*), Xin-Wu Pei *et al*. (2005) introduced an HL gene, driven by a constitutive cauliflower mosaic virus 35S (CaMV35S) promoter into the banana via *Agrobacterium*-mediated transformation. PCR confirmed that transgenic plants were obtained. The development of Panama wilt symptoms were examined after the plants had been grown in pots. The type of explant was an important factor affecting the transformation of banana plants with the HL gene. When corm slices were used as explants, the transformation efficiency was lower

than when embryogenic cell suspensions were used. The authors concluded that *Agrobacterium*-mediated transformation, with the assistance of particle bombardment, is a powerful approach for banana transformation and that a transgenic *HL* gene can cause resistance of the crop to FocR4 in the field. This study proved that HL inhibited the hypha growth of FocR4, currently the most virulent race of Panama wilt that leads to disastrous banana disease.

An East African highland banana cultivar "Gros Michel" (GM) has been transformed with two rice chitinase genes (rcc2 and rcg3). It is known that chitinases are involved in defense mechanisms, because of their anti-fungal properties. After molecular and biochemical characterizations, all transgenic GM lines will be field-tested under confined conditions in Uganda, at the National Agricultural Research Organization (NARO). Chitinase expression will be monitored in different environments (greenhouse and field conditions) to have a better insight in the temporal stability of transgene expression. In the longer term, tissue-specific expression of rice chitinases will be studied by using banana promoters isolated via gene tagging. Transformation of sterile triploid dessert banana cultivars could certainly have a significant commercial impact. Success in these endeavours will lessen the required fungicide inputs for banana cultivation, and will improve fruit shelf-life in the markets.

7.3.4 Virus resistance

Engineering resistance to banana bunchy top virus (BBTV) is another important objective for banana transformation, since no natural resistance to this virus has so far been identified in the *Musa* gene pool (Sagi *et al.,* 1998). One of the strategies now being tested in several laboratories is the expression of various BBTV genes in transgenic banana plants in order to interfere with the normal replication, encapsidation or movement of the virus. Another approach is the expression in bananas of heterologous antiviral proteins that are known to act by the inhibition of viral replication or translation. Similar strategies can also be considered against another recently emerged DNA virus, the banana streak virus.

7.3.5 Nematode resistance

Transformation of EAHB banana plants showing resistance to nematodes has also been attempted. The highest level of expression was obtained when cystatin from chicken egg white was used (Fig. 2).

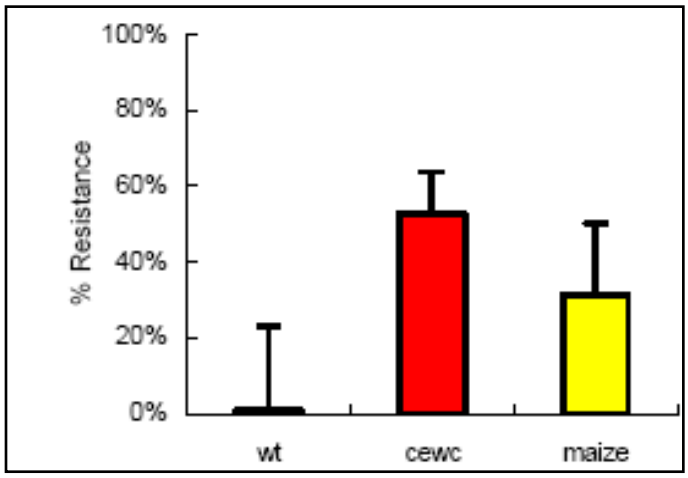


Fig. 2: Partial resistance of two transgenic lines of East African Highland banana to *Meloidogyne incognita* expressing either chicken egg white cystatin (CEWC) or maize cystatin in comparison to the wildtype (wt) of the same cultivar (*Source: Vain, P and Shape, J. PSRP Project R8031 March 2006*)

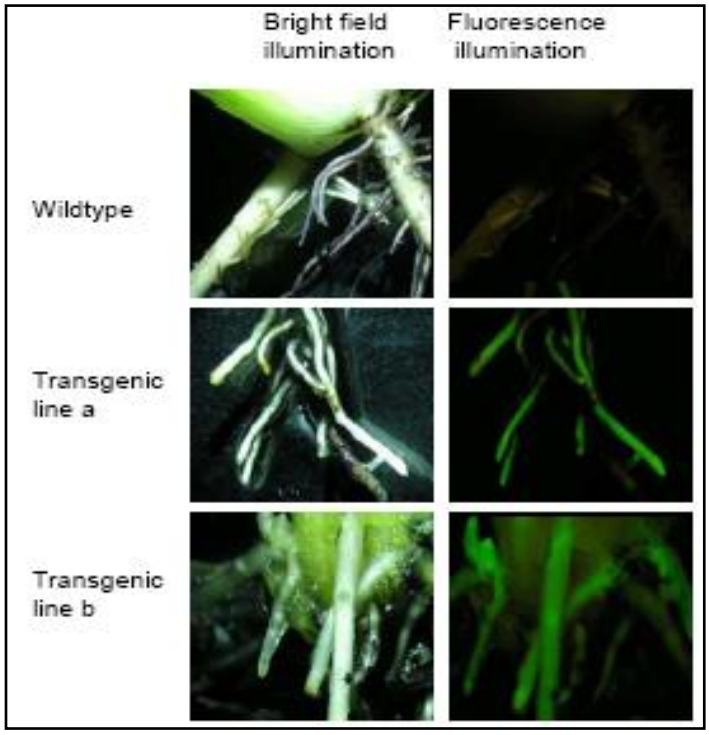


Plate 9: Expression of a fluorescent reporter gene encoding green fluorescent protein in East African Highland banana (*Source: Vain, P and Shape, J. PSRP Project R8031 March 2006)*

Transformation was achieved at the John Innes Centre (JIC) by Dr. Philippe Vain. This work provided the first demonstration that East African Highland banana could be transformed for resistance against nematodes. Initial transformation protocols were first established using green fluorescent protein (GFP) as reporter that could be easily visualised (Atkinson *et al*.,2005) (Plate 9). Once banana transformation had been achieved with GFP, further lines were produced by biolistic transformation using Leeds constructs harbouring CaMV35S-Chicken egg white cystatin and CaMV35S-maize cystatin promoters. Plants shown by PCR to carry the gene of interest were evaluated further for expression of mRNA (RT-PCR) and protein. Transgenic lines that expressed the protein were challenged by *M. incognita* in containment (*PSRP Project R8031*, 2006).

The field trial testing on the Cavendish banana lines in Uganda has not yet been done because necessary approval has to be obtained from the various collaborators and other stakeholders (*PSRP Project R8031*, 2006). The lines made in the *PSRP Project (R8031*, 2006) programme have only a small amount of resistance to *M. incognita* (~50% in the best line). The transgenic lines have been multiplied at JIC (John Innes Centre, UK) and are being evaluated for their resistance to *R. similis* in containment. If resistance over >50% is obtained they will be transferred to NARO (National Agricultural Research Organization), in Uganda. The lines will be used as prototypes and will help establish biosafe practises for transgenic field trials in Uganda if authorised by their National Biosafety Committee (NBC).

In summary, the development of transgenic EAHB bananas that are resistant to BXW is one the initiatives being undertaken by the IITA station at NARO (National Agricultural Research Organization), Kawanda, Uganda. Other partners involved in the development of disease and pest resistant EAHB transgenic bananas include INIBAP (The International Network for the Improvement of Banana), the KUL (The Catholic University of Leuven, Belgium), CIRAD; University of Pretoria (S. Africa) and Leeds University,UK. (Eicher *et al.*, 2006).

SECTION IV

8.0 DEVELOPMENT AND DEPLOYMENT OF TRANSGENIC BANANA IN SUB-SAHARAN AFRICA: AN ENVIRONMENTAL RISK ASSESSMENT

Owing to the devastating effects of banana bacterial wilt in the Great Lakes region, the virtual absence of resistant germplasm and the difficulty of breeding *Musa* conventionally, AATF and a consortium of partners have launched an initiative to genetically modify EAHB for resistance against BBW. The gene of interest is a ferredoxin-like amphipathic protein (*pflp*, formerly called AP1) isolated from the sweet pepper, *Capsicum annuum* (Lin *et al*., 1997). The method of transformation is by use of *Agrobacterium tumefaciens.* While this effort is commendable, there is need for environmental risk assessment to be done to determine the likelihood of transgene escape. This is in line with the recommendations of the Cartagena Protocol on biosafety. The objective of risk assessment, under this protocol is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely receiving environment, taking into account risks to human health.

There is a multitude of concerns about the impact of GM crops on the environment. Key issues in the environmental assessment of GM crops are putative invasiveness, vertical or horizontal gene flow, other ecological impacts, effects on biodiversity and the impact of presence of GM material in other products (Conner *et al.,* 2003). Some groups have expressed concern that widespread use of plants engineered for specific types of pest resistance could accelerate the development of pesticide-resistant insects or have negative effects on organisms that are not crop pests. Another environmental concern is that transgenic, pest-protected plants could hybridize with neighbouring wild relatives, creating "superweeds" or reducing genetic biodiversity.

Transgenes conferring pest or disease resistance could potentially confer a selective advantage on a crop plant and make it more persistent on agricultural land and more invasive in wild habitats. The transgenes could similarly confer a selective advantage if transferred to related wild plant species. Another aspect that needs to be considered is the effect of the resistance genes on pest and pathogen populations. If the transgene provides a very efficient defence it is possible that the pest or pathogen will rapidly become resistant. This is a phenomenon that is well known in conventional plant breeding and arguably has more to do with devising a sound agricultural strategy than assessing risk. The possibility of using the same resistance gene in a range of different crops by transformation means that this prospect has to be taken seriously. A crucial component for a proper assessment is defining the appropriate baseline for comparison and decision. For GM crops, the best and most appropriately defined reference point is the impact of plants developed by traditional breeding techniques (Conner *et al*., 2003). The latter is an integral and accepted part of agriculture.

Environmental risk assessments should take into account the biology of the recipient plant, the characteristics of the introduced genetic material, the properties and consequences of the genetic modification, the scale of release and the evaluation of any risk to the receiving environment that might arise from the release of the GMO. Examples of possible interactions between a GM plant and its environment including potential impact on other organisms are:

- Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations;
- Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;
- Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine;
- Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

8.1 Specific Information for Risk Assessment

The characteristics of the introduced genetic material should include molecular analysis of the introduced genetic material as described in 8.1.1. Phenotypic analysis for agronomic traits using a suitable comparator is also required in order to determine the genetic stability of the introduced genes.

8.1.1 Molecular characterisation

8.1.1.1 Information on the donor and recipient organisms

AATF and partners will be required to provide information both on the organisms used as the DNA donor(s) for genetic modification and the recipient organism. This information should include the most recent taxonomic classification including the family, genus, species, subspecies, cultivar/breeding line or strain.

8.1.1.2 Method used for the genetic modification

The transformation protocol should be described in detail and relevant references for the transformation method should be provided. For *Agrobacterium*-mediated transformation, the strain designation of the *Agrobacterium* used during the transformation process must be provided, including an indication of if, and how, the Ti/Ri plasmid based vector was disarmed. For transformation methods that involve the use of helper plasmids, a detailed description of these plasmids should be given.

8.1.1.3 Information on the DNA used in transformation

A physical and genetic map should detail the position of all coding and non-coding sequences, origins of replication and transfer, and other plasmid elements together with the selected restriction sites for the generation of probes, and the position and nucleotide sequence of primers used in PCR analysis.

A table identifying each component, its size, its origin and its role should accompany the map. The complete sequence of the DNA used in the transformation should be given.

The map/table should also indicate if there have been modifications that affect the amino acid sequence of the product of the introduced gene.

8.1.1.4 Information on the sequences actually inserted/deleted

The project will be required to provide information on:

- The copy number of all detectable inserts, both complete and partial;
- The organization of the inserted genetic material at the insertion site including relevant sequence data of the inserted material and of the surrounding region;
- The sequences flanking the insert should be determined to identify the formation of potential chimeric open reading frames (ORFs) generated at the junctions of the insert and the plant DNA. If a chimeric ORF is identified then expression analysis should be performed to determine if there is potential transcription;
- Potential effects arising from the insertion cannot be characterized by molecular techniques alone but requires a broader consideration including compositional and phenotypic analysis;
- All sequence information including the location of primers used for detection. The above information will also be required when genes have been stacked by the interbreeding of GM lines containing transformation events approved through the regulatory process.

The need for further risk assessment will depend, on a case-by case basis, on the nature of the genetic modifications.

8.1.1.5 Information on the expression of the insert

Partners in this project should be aware that the information on the expression in the plant of genetic elements from any part of the inserted DNA is required if a potential risk is identified. Such requests may be made even where the gene is under the control of a bacterial promoter. Where tissue-specific promoters have been used, information may be requested on expression of target genes in other plant parts relevant for risk assessment. Evidence should be provided to indicate that expression of the inserted gene(s) is as expected and stable in the tissues

targeted. Any expression of potential fusion proteins should be determined. Bio-informatic analysis can be deployed to help identify potential novel fusion proteins. Expression analysis could then be used to detect novel transcripts if identified through the bio-informatics analysis.

The selection of appropriately validated analytical approaches is an issue when applying for regulatory approval. Applications to date have commonly used approaches such as Northern blotting and RT-PCR. The sensitivities of techniques employed will vary and lack of a distinct transcript signal does not necessarily indicate that the corresponding protein is not accumulated. Immunochemical determination by ELISA has proven adequate for determining the levels of novel gene products in the genetically modified plants, while Western blotting provides additional information on the molecular weight of the gene product. Where ELISA tests are routinely used to quantify the expression level of the target protein, it is imperative that the specificity of the antibodies developed is validated. For example, where crude plant extracts are used as test material there may be non-specific cross-reaction with protein other than the target protein.

8.1.1.6 Information on inheritance and stability

AATF and its partners will be required to provide data subjected to statistical analysis from a representative number of generations (vegetative or generative propagation) that demonstrate the inheritance pattern and the stability of the sequences inserted in banana and expression of corresponding proteins. Normally a minimum of three generations is recommended.

8.2 Comparative analysis

8.2.1 Choice of the comparator

In the case of vegetatively propagated crops such as banana, comparative analyses should include the parental variety used to generate the transgenic lines.

8.2.2 Field trials

Protocols of field trials performed with genetically modified and control crops must be specified and documented with respect to:

8.2.2.1 Number of locations, growing seasons, geographical spreading and replicates;

The basic set of data should be obtained from a comparison of the GM banana plant and an appropriate control line grown in the same field under comparable conditions. This comparison should cover more than one growing season and multiple geographical locations representative of the various environments in which the GM plants will be cultivated. The number of replicates at each location should reflect the inherent variability of the plant.

8.2.2.2 Statistical models for analysis, confidence intervals;

Experimental design should be rigorous and analysis of data should be presented in a clear format. Field trial data should be analysed statistically, using appropriate statistical tools. A completely randomised design, for example, could indicate whether the experimental factors (location, year, climatic conditions, and plant variety) interact with one another. The confidence intervals used for statistical analysis should be specified (normally 95%, with possible adjustment according to the hazard of the constituent to be compared).

8.2.2.3 The baseline used for consideration of natural variations;

Data demonstrating the natural range in component concentrations found in non-GM counterparts should be provided to enable additional comparisons with the GM plant in question gathered by the researcher or compiled from literature. The databases that were used for comparison have to be specified. Special attention has to be paid to the comparability of the analytical methods used to create the data. Ranges as well as mean values should be reported and considered. Statistically significant differences in composition between the modified crop and its traditional counterpart grown and harvested under the same conditions should trigger further investigations as to the relationship with the genetic modification process. Modifications that fall outside normal ranges of variation will require further evaluation to determine any biological significance.

8.2.3 Selection of compounds for analysis

Analysis of the composition of the GM plant/food/feed is crucial when comparing the product with its non-GM counterparts. Analysis should be carried out on the raw agricultural commodity, such as banana fruit, as this usually represents the main point of entry of the material into the food/feed chain. Analysis on specific derived products should be required only on a case-by-case basis and when justified scientifically. In each case, key macro- and micro-nutrients, toxicants, anti-nutritional compounds, and other constituents (including moisture and total ash) should be determined:

- Key nutrients are those components that have a major impact on the diet, *i.e.* proteins, carbohydrates, lipids/fats, fibre, vitamins and minerals;
- The vitamins and minerals selected for analysis should be those which are present in nutritionally significant levels and/or that make nutritionally significant contributions to the diet at the levels at which the plant is consumed;
- The specific analyses required will depend on the plant species examined, but should include a detailed assessment appropriate to the intention of the genetic modification, the considered nutritional value and use of the plant. For example, a sugar profile should be included for carbohydrate rich plants like the banana;
- Measures of plant cell wall components are also required for the vegetative parts of plants used for feed purpose. Key toxicants are those compounds, inherently present, whose toxic potency and levels may harm human/animal health. The concentrations of such compounds should be assessed according to plant species and the proposed use of the food/feed product. Examples would include digestive enzyme inhibitors and those anti-nutritional, potentially toxic, or allergenic compounds recognized as being normally present, or newly introduced as a result of the genetic modification. Compounds other than key nutrients and toxicants may be included in analyses on a case-by-case basis.

8.3 Agronomic traits

Unintended effects may also occur during genetic transformation. Traits such as yield and the ability to withstand biotic and abiotic stresses should be evaluated between the GM banana vis a vis the conventionally bred type.

8.3.1 Impact on non-modified crops

The potential consequence arising from out-crossing to other crop cultivars should be considered and assessed for environmental risk. This will vary with crop and there is no risk in banana.

8.3.2 Geographical relevance of data

Data should be provided from field experiments in areas representative of those geographical regions where the GM banana plant will be grown commercially in order to reflect relevant ecological, meteorological, soil and agronomic conditions.

8.3.3 Will GM crops lead to superpests and superdiseases?

The release and widespread cultivation of GM crops with pest or disease resistance has raised concerns that this will impose intense selection pressure on pest and pathogen populations to adapt to the resistance mechanism. This might result in the development of superpests and superdiseases that would be difficult or impossible to control (Conner *et al*., 2003). The development of GM lines, isogenic for the presence/absence of various genes targeting specific pests or pathogens, offers a new opportunity for more efficiently implementing multi-line strategies and durable approaches to minimise the “breakdown” of pest and disease resistance genes in crops.

The history of plant breeding has clearly established that pest and pathogen populations can quickly adapt to crop cultivars with new resistance genes (e.g. Bonman *et al*., 1992; McIntosh and Brown, 1997). The use of host plant resistance genes has been extensively used for pest and disease control in breeding programmes of many crop species, especially in cereal crops. Although many resistance genes have been identified in crop germplasm, there has been no easy way to predict the quality or durability of these resistance genes (Leach *et al*.,

2001). The “breaking down” of disease resistance genes is usually associated with qualitative resistance conferred by single major genes (R genes), where resistance versus susceptibility results from a gene-for-gene interaction between the R genes in the host and avirulence genes in the pathogen (Flor, 1971). The resistance conferred by many R genes has not been durable as a consequence of rapid changes in pathogen populations (Leach *et al*., 2001). The most widely cited examples of durable resistance against bacterial or fungal pathogens have involved supposedly complex, multigenic quantitative traits (Johnson, 1984; Parlevliet, 2002). However, there are examples where single R genes have conferred highly durable resistance, e.g. the Lr34 gene conferring resistance to leaf rust in wheat (Kolmer, 1996) and the Xa4 gene conferring bacterial blight resistance in rice (Bonman *et al*., 1992).

8.4 Gene Flow

The issue of whether gene flow can occur between transgenic plants and their wild/feral relatives is of major concern. Three major ecological risks are recognized including (i) invasiveness of the transgenic plants (ii) invasiveness of the transgene itself and (iii) effects on non-target organisms. Moreover, development of resistance or increased tolerance by the target organisms can also have adverse impacts to the environment.

8.4.1 Impact on agricultural and natural ecosystems

There are concerns that the release of GM crops will result in such plants becoming agricultural weeds and, therefore, add to the already large agricultural weed management burden of farmers. It is also feared that such plants may invade natural habitats and, as a consequence, compromise their biodiversity values (Conner *et al*., 2003). However, modern cultivars are highly unlikely to revert to weedy derivatives, with or without further genetic modification. This is because the cultivars no longer possess weedy characteristics such as seed dormancy, phenotypic plasticity, indeterminate growth, continuous flowering and seed production, and seed dispersal (Baker, 1965; 1974), which have been bred out of the most important crop plants over thousands of generations. Therefore the

ability of cultivated crops to become weeds has been severely retarded in the absence of gene introgression from the wild races (Conner *et al*., 2003). The rare occurrence of spontaneous "weedy types" within crop fields is usually associated with chance hybridization with wild races during seed production (Wijnheijmer *et al*., 1989).

Will transgenic banana crops invade agricultural and natural ecosystems?

In the case of banana, hybridization with wild relatives is unlikely to occur due to sterility of the cultivated types. Therefore, genetically modified banana crops are no more likely to become weeds outside farming situations than other conventionally bred cultivars have in the past (Conner *et al*., 2003). In order to assess the potential weediness of a transgenic crop the key issue is invasiveness. When assessing the invasiveness potential of GM crops the key issue to address is whether their weedy characteristics are likely to be different when the expression of the transgene is taken into account.

8.4.2 Impact on wild plants

Concerns have been expressed that GM crops will hybridize with related species and result in the introgression of transgenes to weedy relatives. For transgenes conferring resistance to pests, diseases, and herbicides it is often suggested that this may result in enhanced fitness, survival and spread of weeds (Ellstrand, 2001). This too has the potential to add to the agricultural weed management burden by farmers, and/or may result in further invasion of natural habitats and compromise the biodiversity values of these habitats. The potential for a crop to hybridize with a weed is highly dependent on sexual compatibility and relatedness between the parent species. The sterility of cultivated triploid banana makes it incompatible with wild relatives.

Gene introgression from one species to another or from a crop to a weed of the same species also requires repeated backcrossing to effect the incorporation of alleles from the gene pool of one population to another recipient population. The key issue of whether gene introgression can occur from a crop to a weed is the

fitness of any possible hybrid populations and their persistence through several generations. Such fitness is based on the cumulative effects of all the above factors, with a poor performance at any step severely limiting gene introgression to weeds (Conner *et al*., 2003).

Plant breeders have released many cultivars with new genes for resistance to pests, diseases and environmental stress over many years. Any impacts resulting from the introgression of such traits into weedy species are equally likely for the products of plant breeding and genetic modification. The risks are no different and the uses of resistance genes in cultivars from traditional breeding have not been noted to enhance the survival and spread of weeds during the past history of crop breeding (Conner *et al*., 2003). The potential consequence arising from out-crossing to compatible wild species should be considered and assessed for environmental risk.

Will transgenes from banana outcross to other species and increase weediness?

Bananas are cultivated vegetatively hence such a risk is very minimal therefore natural hybridization is an unlikely event.

8.4.3 Impact on organisms and ecological processes

Risk assessments should be carried out for each of the different functional environmental compartments that are exposed to the GM banana plant. These should include:

- Whether any parts of the GM banana will remain in the environment after harvest. This will depend on the management regime or agronomic practices of the banana crop;
- Soil fertility studies as they strongly influence the growth and productivity of plants. The risk assessment should aim to establish if direct or indirect effect(s) of the genetic modification in the GM banana plant have any long-term or sustainable deleterious effect on the recognized soil microbial communities and the associated functional activities that are responsible for

maintaining the agronomically relevant processes of soil fertility and plant productivity;

- The assessment should also address the fate of any (newly) expressed substance(s) in those environmental compartments where the GM bananas are introduced and which result in exposure of non-target organisms (e.g. in soil after the incorporation of plant material);
- Risk assessment should also include an analysis to determine if a shift occurs in populations of organisms in the presence of the modified plant.

Exposure should also be estimated to soil organisms and decomposition function (e.g. earthworms, micro-organisms, leaf litter breakdown) in relation to potential transfer to soil micro-fauna and impact on degradation.

An assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches:

- The project partners should describe the appropriate commercial management regime for the banana GM crop including changes in pesticide applications, rotations and other crop protection measures where different from the equivalent non-GM crop under representative conditions;
- The project partners should aim to assess the direct and indirect, immediate and delayed effects, of the management of the GM banana crop on all affected habitats. This should include the biodiversity within the GM crop and adjacent non-crop habitats.

The necessary scale of such studies will depend on the level of risk associated with the banana bacterial wilt resistance gene being introduced and on the quality and extent of the available literature that is relevant to the particular risk assessment.

8.4.4 Horizontal gene transfer (HGT)

Horizontal gene transfer (HGT) is defined as the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Gay, 2001). HGT between bacterial species is

particularly common when it involves plasmids and transposons (Courvalin, 1994; Landis *et al*., 2000; Lorenz and Wackernagel, 1994). The general concern with respect to GM crops is that the novel genes in such crops will result in a transfer of that material to other species and cause harm. Of particular concern are putative recipient micro-organisms in soil or in the digestive track of humans and livestock (Dröge *et al.*, 1998; 1999). The initial debate on HGT from GM crops focused on the presence of antibiotic marker genes in the plants. Although kanamycin ang hygromycin are of limited therapeutic use, the EU has recommended their phasing out from commercial cultivars (Conner *et al*., 2003).

In the environment, HGT could affect the soil microflora and create novel pathogens, or have other influences detrimental to either agricultural productivity or biodiversity. The most popular technology of gene transfer to plants using *Agrobacterium tumefaciens* is based on HGT. Whereas the mechanisms of HGT from *A. tumefaciens* to plant cells is known in considerable detail, there is no known mechanism for HGT from plants to other organisms (Conner *et al*., 2003). Several experimental studies have been published that all failed in demonstrating HGT from transgenic plants to bacteria (Bertolla and Simonet, 1999; Gebhard and Smalla, 1999; Schlüter *et al*., 1995).

Will GM banana crops contribute to horizontal gene transfer?

Since there are no documented studies on horizontal gene transfer (HGT) from transgenic plants to bacteria, such an event is unlikely to occur between transgenic banana and bacteria, and further studies/research may be necessary.

8.4.5 Effect of GM plants on non-targets

Insect species that may be affected directly by GM crops is the honey bee (*Apis mellifera*), a beneficial insect which collects pollen and is therefore heavily exposed. A number of studies have investigated the possible impacts of GM plants and purified recombinant proteins on bees. Direct toxicity is extremely rare and evidence from the most widely grown commercial crops has found no effect on colony performance (e.g. Malone and Pham-Delegue, 2001). At high doses, serine protease inhibitors, however, have been shown to inhibit bee gut proteases, which may result in reduced adult longevity (Malone *et al*., 2000). However, the expression level in pollen from GM plants is not likely to reach the high dose required. In one study, pollen expression of cowpea trypsin inhibitor (CpTI) reduced the ability of bees to learn a conditioned response to floral odour (Picard-Nizou *et al*., 1997), although other studies involving the expression of two other serine proteases (Girard *et al*., 1998) or the cysteine protease inhibitor, oryza cystatin (Girard *et al*., 1998; Jouanin *et al*., 1998), found no effects on learning or foraging behaviour of bees. Overall, the ecological relevance of such effects, if any, in agricultural fields or beyond is unlikely to have any further undesirable or important consequences.

Even more difficult to study are any indirect effects on non-target insects (or other organisms) via the so-called multi-trophic food chains. Investigations of multi-trophic effects were initiated with studies of the impact of Bt GM plants on predators and parasites of the pest insect targeted by Bt. For example, field data from insect-resistant GM crops expressing *Cry* genes have failed to find impact on predator numbers between GM-Bt cotton and non-GM cotton and, in some cases, numbers have increased in GM plots (Schuler *et al*., 1999a). When direct toxicity has not been found in the laboratory to be due to either purified toxin or GM plant material, it would be unexpected to find any ecological effect from field use.

Impact should be assessed on non-target arthropods, grazing birds and mammals. Such studies should include laboratory, greenhouse or field exposure experiments set up in such a way that enough statistical power is obtained to be able to

observe possible negative impacts on non-target organisms. This risk assessment should take account of where in the plant and to what degree the inserted genes are expressed and therefore the extent to which non-target organisms are exposed either directly or indirectly. Data on the comparative susceptibility of the GM banana plant to pests and diseases compared with that of the non-modified plants are useful indicators of effects together with observations on agronomic performance during greenhouse and experimental field trials.

8.4.6 Effect on soil organisms

Concerns about the secondary ecological impacts of GM crops are starting to focus on soil ecosystems. The potential impact of any GM plant on soil organisms includes potential toxicity to a range of organisms (most of which are not tested under standard conditions, as many can not be cultured), the persistence in soil of any transgene product with undesirable effects, and the likelihood of such products ending up in soil. Rhizosphere microbes are particularly exposed to decomposing plants and exudates from GM plant roots. Many studies have reported no changes in microbe populations in the rhizosphere over a range of many different GM plant modifications (e.g. Griffiths *et al*., 2000, for lectin-producing potatoes). In a few cases, changes in the populations of bacteria, fungi and soil invertebrates have been detected, even though no direct toxicity to the organisms has ever been demonstrated (Conner *et al*., 2003). Harris *et al*., 1999 reported that risk assessment of the impact on soil organisms, of transgenic banana harbouring the cystatin gene and conferring resistance to nematodes was under investigation. It will therefore be interesting to know their findings which will help other researchers to formulate their risk assessment strategies.

8.5 Risk Assessment of genetically modified banana in East Africa

Africa is considering introduction of transgenic food crops as well as cash crops. A case in point is the ongoing field trial of virus resistant transgenic sweet potato and development of insect resistant Bt maize in Kenya. However, many scientists have long expressed concerns against potential environmental impacts of wide-spread use of transgenic crops.

Using taxonomy, ecology, distribution frequency and dispersal/gene flow of the host plant and its wild/feral relatives, it is possible to determine the probability of the transgenes escaping from human confines. More specific is the information on presence and sexual compatibility with wild/feral relatives, mode of reproduction and dispersal. Through a system developed by Dutch scientists (Frietema De Vries *et al*., 1992) for Netherlands and later adopted in Switzerland (Jacot and Ammann 1999, Ammann *et al*., 2001), which classifies crops into risk categories on basis of probability of gene flow through pollen or diaspores dispersal, we considered real chances of gene flow from cultivated banana to its wild/feral flora in the region. Gene flow can only occur either through dispersal of diaspores (e.g. seeds) or hybridisation with related species (pollen dispersal). Moreover, effective dispersal of pollen or diaspores depends on the distribution frequency of a crop and its wild/weedy relatives in the region. Therefore, the probability for gene flow from transgenic plants in a region can be estimated through assessment of the three floristic factors vis-à-vis pollen dispersal, diaspores dispersal and distribution frequency.

Since most banana cultivars do not produce seeds under natural conditions, crosses with other varieties or species will not occur. In these cases, the introduced gene remains confined to the variety in which it has been introduced. The gene flow indices for banana in the Great Lakes region are relatively low and the chances of negative impacts of GM banana running wild are minimal and there is no significant impact on environment. All East African highland bananas are sterile triploids and therefore difficult to improve through cross-breeding. With three genomes, as compared to two or four, no pollen is produced and plants are sterile (Smale and de Groote, 2003)**.** Though professional breeding of bananas began during the 1920s, it was not until recently that a major breakthrough was achieved through the development of a hybridization technique advanced by the Fundacion Hondurena de Investigacion Agricola (FHIA) and employed by breeders at the International Institute for Tropical Agriculture (IITA) in Uganda. Male fertile diploids

are used to pollinate triploid varieties to produce tetraploid hybrids, which can then be crossed. The hybrid tetraploid expresses the traits of both parents.

8.6. Regulation of transgenic crops

In every country, the prescribed biosafety requirements are to be fulfilled before a transgenic product is approved for commercialization. With respect to international obligations, a majority of African countries are signatories to and/or have ratified the Cartagena Biosafety Protocol, an addendum to the Convention on Biological Diversity, which governs safe transboundary movement of living modified organisms, among other provisions for ensuring safety in biotechnology (Eicher *et al*., 2006).

Biotechnology tools range from tissue culture to molecular breeding and genetic engineering. Although many African countries have made impressive progress in biotechnology and biosafety frameworks, only four countries in the region, namely South Africa, Zimbabwe, Egypt and Malawi have legal mechanisms for biosafety. The rest are still at varying stages in the development of their biosafety systems. GM crops are currently grown commercially in only one country in Africa namely South Africa (Eicher *et al*., 2006).

To implement a national biosafety system, it is important for countries to identify the goals and objectives of their system and the existing context for biotechnology and biosafety oversight. In Kenya for example, the National Biosafety Committee (NBC) under the National Council for Science and Technology (NCST), is mandated to regulate research and field testing of GM crops. This is in line with Catargena Protocol on Biosafety that requires every member country that is party to the Convention on Biological Diversity to have a Biosafety Clearing House (BCH). Uganda and Tanzania also have National Biosafety Committees to implement their Biosafety regulations.

SECTION V

9.0 PUBLIC HEALTH RISKS (FOOD/FEED SAFETY ASSESSMENT)

Despite the benefits of genetic engineering, there are concerns about whether recombinant DNA techniques carry greater risks than traditional breeding methods. Consumer acceptance of food derived from genetically engineered crops has been variable. Many individuals express concerns regarding the environmental impact and ethics of the new technology, and about food safety. Other principal concerns are that transgenic foods will be toxic or allergenic.

The evaluation of public health risks concerning food/feed safety seeks to establish that new transgenic varieties are as safe as crops produced by conventional methods. This requires extensive testing involving the evaluation of the safety of the newly added DNA; the new gene product and the overall safety of the balance of the food. A concept called “Substantial Equivalence” guides the assessment. The principal food safety issues for new varieties of crops are:

1) The potential toxicity of the newly introduced protein(s);
2) The potential changes in allergenicity;
3) Changes in nutrient composition;
4) Unintended effects giving rise to allergenicity or toxicity and;
5) The safety of antibiotic resistance marker-encoded proteins included with the transgene.

All of these must be taken in the context of the predicted range of dietary exposures.

Frank-Oberaspach and Keller (1997) reviewed the consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. They reported on many classes of actual and putative toxins and allergens, concluding that several naturally occurring defence substances found in plants are highly toxic to mammals, but also indicating that food safety can be severely influenced by natural pathogens and their products. It is interesting how little we yet know about the toxicity of non-engineered foods. Known toxins and allergens can be screened for in advance, however, to reduce the chances of releasing potentially dangerous

foods. Careful labelling of products would be informative for customers with allergies and for those averse to buying a product derived from a transgenic crop.

Could the presence of antibiotic resistance genes in GM crops enhance existing problems with drug-resistant bacteria in human therapy?

Bengtsson (1997) maintained that as some crop varieties will be transformed many times, antibiotic resistance genes will accumulate, and it is therefore sensible to remove them as plant breeders will soon encounter difficulties in locating new, harmless antibiotic marker genes. The obvious fear is that antibiotic marker genes could be recruited into humans (and domestic animals) rendering antibiotics ineffective in curing bacterial infections. Technologies targeting marker gene removal (incorporating site-specific recombinases) have been developed (Kilby *et al*., 1993) and alternative marker genes have been developed. Although the World Health Organisation (WHO) has judged antibiotic marker genes to be safe (WHO, 1994), the outcome of their use might be hazardous if they represent a major source of resistance to a wide class of antibiotics.

When it became apparent that antibiotic selectable markers could be removed from GM plants and/or alternative selectable markers had been developed for plant transformation, the HGT debate shifted to involve all transgenes in GM plants (Conner *et al*., 2003). Issues such as whether HGT could affect the intestinal microflora upon consumption of GM crops or whether HGT could transform intestinal cells and change their phenotype have been raised.

In addition to the molecular characterization of the genetically modified plants and the necessary comparative compositional data to assess the extent of equivalence, further information is needed for the safety assessment of material intended for use as human food or animal feed.

9.1 Product Specification

AATF and its partners will be required to provide information on origin and composition of the GM banana plant and GM food/feed to ensure the identity between the product tested/evaluated and the product to be marketed. In the design of the specification, parameters most relevant for the characterization of the product from a safety and nutritional point of view should be considered. Information on the availability of specified reference material should be submitted.

9.2 Effect of the production process

For processed foods/feeds derived from GM sources a description of the production process should be provided which should comprise a general outline of the processing steps and a detailed description of the conditions applied (description of physical, chemical and biochemical parameters). It is important to assess if, and to what extent, the processing steps lead to the concentration or to the elimination, denaturing and/or degradation of DNA and the novel protein(s) in the final product.

9.3 Anticipated intake/extent of use

The concentrations of the new gene products and constituents produced, or modified by the intended genetic modification (*e.g.* due to changes in metabolic pathways) in those parts of the GM plant intended for food or feed use, should be determined by appropriate methods. Expected exposure to these constituents should be estimated taking into account the influences of processing, storage and expected treatment of the food/feed in question. If possible, particular sections of the population with an expected high exposure should be identified and this should be considered within the risk assessment.

9.4 Toxicology

The toxicological requirements for food and feed derived from GM banana plants must be considered and will be determined by the outcome of the assessment of

the biological significance of any differences identified between the GM product and its conventional counterpart. This would not only include studies on newly expressed proteins but also the consequences of any genetic modification (e.g. gene silencing or over-expression of an endogenous gene). In principle, the safety assessment must consider the presence of proteins expressed as result of the genetic modification, the potential presence of other novel constituents and/or possible changes in the level of natural constituents beyond normal variation

9.4.1 Information on natural food constituents

Natural food constituents comprise a large variety of substances: macro- and micronutrients, secondary plant metabolites as well as natural toxicants and anti-nutritional factors. If the content of such natural food constituents is increased beyond the natural variation, a detailed safety assessment based on the knowledge of the physiological function and/or toxic properties of these constituents should be submitted. The result of this assessment would determine if and to what extent toxicological tests are required.

9.4.2. Testing of the whole GM food/feed

If the composition is modified substantially, or if there are any uncertainties on the equivalence to a traditional counterpart, not only novel constituents, but also the whole GM banana food/feed should be tested. For foods and products that can be used for both food and feed purposes, the testing programme should include at least a 90-day feeding study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. Additional toxicological studies may also be necessary, depending on the potential exposure, the nature and extent of deviation from traditional counterparts and the findings of the feeding study.

9.5 Allergenicity

The intrinsic allergenicity of the foreign protein(s) encoded by the introduced gene(s) must clearly be considered. Moreover, the consequences of any possible unintended effects of the genetic modification on the endogenous allergenic

potential of the plant or plant product should also be considered. For example, unintended qualitative or quantitative changes could occur in the pattern of allergenic proteins naturally present in the conventional banana plant or product.

9.5.1 Assessment of allergenicity of the newly expressed protein

In every case a search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be made as the first step in the assessment. If the source of the introduced gene is considered allergenic, but no sequence homology to a known allergen is demonstrated, specific serum screening of the expressed protein should then be undertaken with appropriate sera from patients allergic to the source material using relevant validated immunochemical tests.

If the source is not known to be allergenic but if a sequence homology to a known allergen is demonstrated, the specific serum screening should be conducted with sera from patients sensitized to this allergen. If the source of the gene/protein is not known to be commonly allergenic and no sequence homology to a known allergen is demonstrated, or if the result of the specific serum screening of a newly expressed protein from a source known to be allergenic is equivocal, additional tests should be performed. These include pepsin resistance tests or targeted serum screening.

9.5.2 Further analyses

Stability to digestion by proteolytic enzymes has long been considered a characteristic of allergenic proteins. Although it has now been established that no absolute correlation exists resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment.

In the case of resistance of a protein to degradation in the presence of pepsin under appropriate conditions, further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. Complementary data on the biological origin and function and structural features of the newly

expressed protein may also be provided in order to increase the facts to support a conclusion.

9.5.3 Assessment of allergenicity of the whole GM plant or crop

If the host of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole GM food/feed should be tested by comparison of the allergen repertoire with that of the conventional non-GM variety.
Data on the prevalence of occupational allergy in workers or in farmers who have significant exposure to GM plant and crops or to the airborne allergens they may contain will provide useful information for the risk assessment process.

9.6 Nutritional assessment of GM food

The development of GM foods has the potential to improve the nutritional status of individuals and populations and provide products with enhanced functionality. GM foods also have the potential to introduce nutritional imbalances as a result of both expected and unexpected alterations in nutrients and other food components.
The nutritional evaluation of GM banana foods should consider:

- nutrient composition;
- biological efficacy of nutrient components in the foods;
- assessment of dietary intake and nutritional impact

This will demonstrate substantial equivalence to an existing non GM banana food. Information on the anticipated intake/extent of use of the GM food will be required and the nutritional consequences should be assessed at average and at upper levels of daily intake. The influences of non-nutrient components of the GM food should also be considered.

9.7 Nutritional assessment of GM feed

In cases where composition of the GM banana plant differs significantly from the non-GM counterpart, a full range of physiological-nutritional studies should be carried out with representative target animals. These studies could include digestibility, balance experiments or the determination of the nutritive value.

For feeds, it is recommended that comparative growth studies are conducted with a fast growing livestock species such as the broiler chick. Because of their rapid weight gain, broilers are particularly sensitive to the presence of toxic elements in their feed. Studies of this type are, however, limited to those materials suitable for inclusion in broiler diets and which can be nutritionally matched to a suitable control diet.

9.7.1 Animal products

The safety of products derived from animals fed a diet containing GM banana plant material and consumed by humans should be considered. However, it is considered unlikely that a potential gene transfer from GM banana plant material to animal cells will result in the expression of heterologous proteins which might be present in animal products or that intact newly expressed proteins will be absorbed. Consequently, it is not considered necessary to test routinely for the presence of introduced genes or their products unless their characteristics suggest cause for concern.

Proteins introduced into the GM plant and known to modify plant metabolism may alter the nature or concentration of metabolites which may have toxicological implications for the animal and/or consumers of animal products. In such cases further studies should be performed with respect to the toxicological implications for the animal and/or consumers of animal products.

In summary food/feed safety assessment is an important component of risk analysis. Transgenic plants like *Bt* maize and cotton that have been commercialized so far have undergone and passed extensive safety trials with regard to potential for food toxicity, food allergenicity, cross pollination and effect on non-target beneficial organisms including biological control agents. Such studies, with the exception of cross pollination will be required for transgenic banana before commercialisation.

SECTION VI

10.0 PUBLIC PERCEPTION AND CONCERNS REGARDING THE USE OF BIOTECHNOLOGY PRODUCTS IN KENYA

10.1 Background

The success of any biotechnology program depends on whether consumers accept its products (Springer *et al*., 2002). To determine the level of awareness on biotechnology and acceptance of genetically modified crops and products in Kenya, a cross section of farmers were interviewed from representative banana growing districts in Western, Eastern, Nyanza and Central provinces of Kenya. A cross-section of consumers, traders and some industry players were also interviewed in Nairobi and a few other selected areas to determine the level of awareness and acceptance of genetically modified crops and products.

10.2 Methodology

A survey of 155 farmers was conducted in eight districts within the four provinces to determine their level of awareness of banana *Xanthomonas* wilt and willingness to use transgenic banana as a control measure. Data was collected from personal interviews in December 2006 using a structured questionnaire. Three border districts thought to be affected by BXW were chosen namely Bungoma, Teso and Busia. From Central Kenya, Maragua, Embu and Meru districts were selected while Kisii Central and Gucha districts were selected from Nyanza province. The divisions affected within each district were chosen with the help of Agricultural Officers. At least twenty farmers were interviewed from two selected locations . Interviews were conducted in five other banana-producing districts that are presumed still free from BXW. Sampling was similar to that in the border districts.

We also sought to obtain information from consumers. A total of 93 consumers were interviewed. The consumers comprised of 24 from the industry and traders category as well as 69 members of the general public. The latter mainly comprised

of direct consumers notably teachers, students, civil servants, politicians, etc. mainly from Nairobi province, with a few from other towns in Kenya. Members of the public were interviewed randomly in open markets, supermarkets, social places and both private and public institutions in several towns in Kenya. Eighteen institutions were selected randomly based on the following criteria: research organisations, regulators, industry and consumer organisations mainly non-governmental organisations.

10.3 RESULTS OF PUBLIC SURVEY

10.3.1 FARMERS

10.3.1.1 Socioeconomic characteristics

The socioeconomic characteristics of farmers surveyed in this study showed clear differences among districts. Maragua had the highest number of men working on farms (90%) followed by Teso (71%). Bungoma had the highest number of women (59%) on farms. The most educated farmers were in Embu where all of them had at least primary education (Table 3). Majority of farmers (84%) are small holders in all the eight districts with an average of 3.13 acres.

10.3.1.2 Main crops

With exception of maize, the main crops varied across the eight districts (see Table 4). Overall, maize which is ranked by 50% of the farmers as the most important crop is followed by banana (18%). Some of the crops were unique to particular districts for instance cassava in Teso (62%), Khat (or Miraa) in Meru North (40%) and coffee in Embu (35%). Banana was ranked first in Maragua district.

Table 3: Farmers' socio-economic characteristics expressed as percentage of respondents in different districts in Kenya

Variable	Category	Bungoma	Busia	Teso	Maragua	Embu	Meru	Kisii Central	Gucha	Total
Gender	Male	40.9	57.1	71.4	90.5	55	65	68.8	64.3	63.9
	Female	59.1	42.9	28.6	9.5	45	35	31.3	35.7	36.1
Education	Informal	4.5	47.6	23.8	14.3	-	20	6.3	-	15.5
	Primary	50	42.9	42.9	38.1	5	40	35.7	33.3	38.7
	Secondary	27.3	4.8	14.3	33.3	65	40	50	53.3	34.2
	College	18.2	-	14.3	9.5	10	-	14.3	13.3	9.7
	University	-	4.8	4.8	4.8	-	-	-	-	1.9
Land size	≤5 Acres	81	81	90	80	90	95	88	93	86
	5.1 - 10 acres	14	19	10	5	10	5	6	-	10
	>10 acres	5	-	-	15	-	-	6	7	4
Mean		3.25	3.11	2.88	3.65	2.65	1.78	3.97	4.2	3.13

Table 4: The main crops grown expressed as percentage of respondents in eight districts and their ranking

Variable	Category	Bungoma	Busia	Teso	Maragua	Embu	Meru	Kisii Central	Gucha	Total
Main crop	Maize	68	81	-	52	-	30	100	100	60
	Banana	-	-	-	48	45	-	-	-	14
	Sugarcane	32	-	-	-	-	-	-	-	5
	Coffee	-	-	-	-	35	-	-	-	5
	Khat (Miraa)	-	-	-	-		40	-	-	5
	Cassava	-	-	62	-	-	-	-	-	11
Banana ranking	First	-	5	-	48	40	10	-	-	14
	Second	-	5	-	24	40	10	47	77	22
	Third	41	24	14	24	10	40	23	25	-
	Fourth	50	38	48	4	5	35	7	-	26
	Fifth	9	28	24	-	5	20	6	-	13

10.3.1.3 Main constraints

Majority of the farmers cited disease as the most limiting factor to crop production. However, this differed among districts with Kisii Central citing disease as the highest (93%) followed by Teso (90%). Pest constraint was ranked second in overall terms with Bungoma (59%) and Embu (38%) being the most affected. Embu and Maragua farmers cited poor soil fertility as an important constraint in their efforts to produce banana (see Fig. 3). In Bungoma, Teso, Kisii Central and Gucha districts, soil fertility was not a problem at 0%.

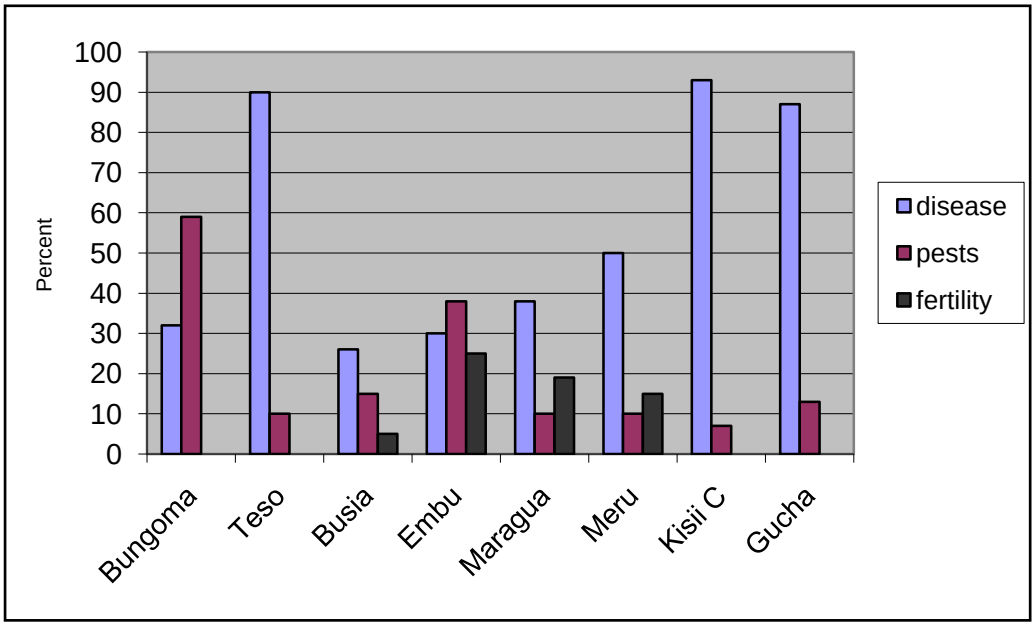


Fig. 3: Main constraints limiting banana production in six districts of Kenya (expressed as percentages within individual districts)

10.3.1.4 Farmers' awareness of banana *Xanthomonas* wilt

Awareness of banana *Xanthomonas* wilt (BXW) among farmers was high in Gucha (100%), Maragua and Kisii Central (81%), Bungoma (77%) and Teso (76%) districts (see Table 5.). However farmers could not definitively name the disease except for a few in Maragua, Meru North and Embu. In Teso (76%) and Bungoma (59%) of farmers were aware of the spread of BXW. In general, awareness of control measures for BXW is very low (9%).

Table 5: Farmers' awareness of the characteristics of banana *Xanthomonas* wilt (expressed as a percentage of respondents in different districts)

Variable	Category	By District							
		Bungoma	Busia	Teso	Maragua	Embu	Meru	Kisii Central	Gucha
Awareness of symptoms	Low	9	62	-	19	95	100	-	-
	Medium	14	19	23	-	5	-	19	-
	High	77	19	76	81	-	-	81	100
Awareness of spread	Low	9	71	10	7	95	29	69	57
	Medium	32	5	14	33	5	-	13	29
	High	59	24	76	10	-	-	19	14
Awareness of control	Low	100	86	86	38	95	95	6	-
	Medium	-	14	5	24	5	-	94	93
	High	-	-	9	38	-	5	-	7

10.3.1.6 Farmers' awareness of biotechnology

Districts close to Nairobi showed a high level of awareness on biotechnology. In Maragua which is closest to Nairobi, 95% of the farmers interviewed were aware of this technology. In contrast, in the far flung border district of Teso, an awareness level on biotechnology was only 33% (see Fig. 4).

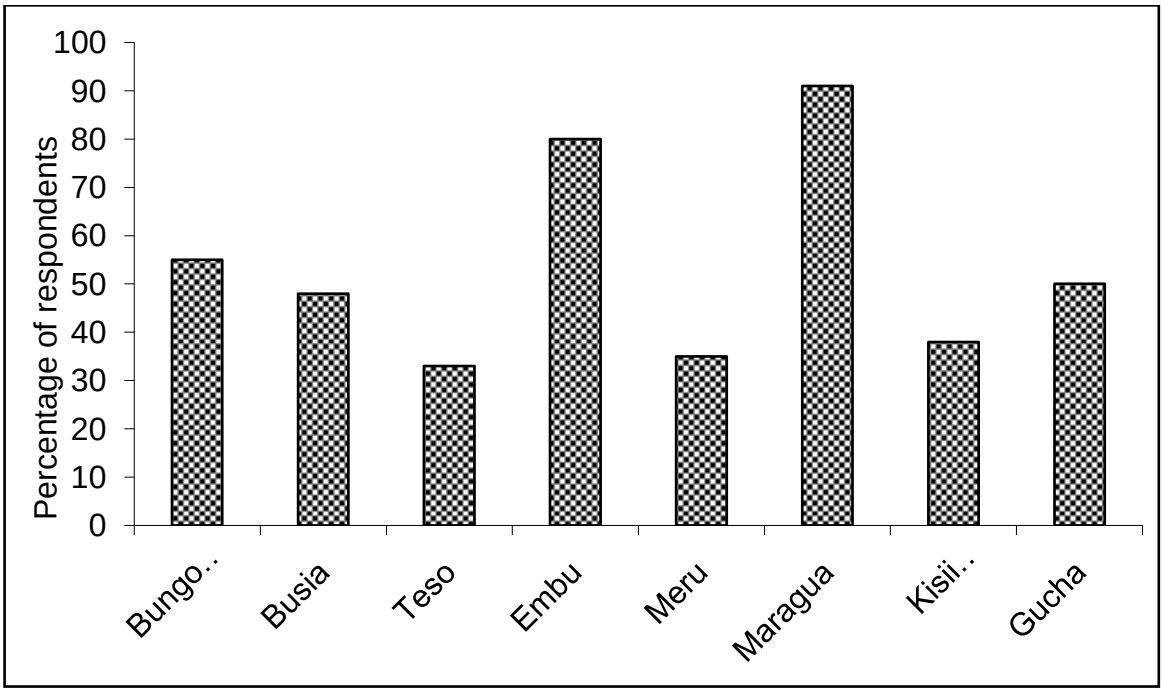


Fig. 4: Farmers` awareness of biotechnology by districts

10.3.1.6 Farmers' willingness to adopt new technologies

An overwhelming majority of the farmers interviewed in seven of the eight districts were willing to adopt new technologies aimed at overcoming the constraints faced during banana production except in Embu (Fig. 5). Interestingly; this is the district with plenty of tissue culture banana material supplied by the Kenya Agricultural Research Institute (KARI).

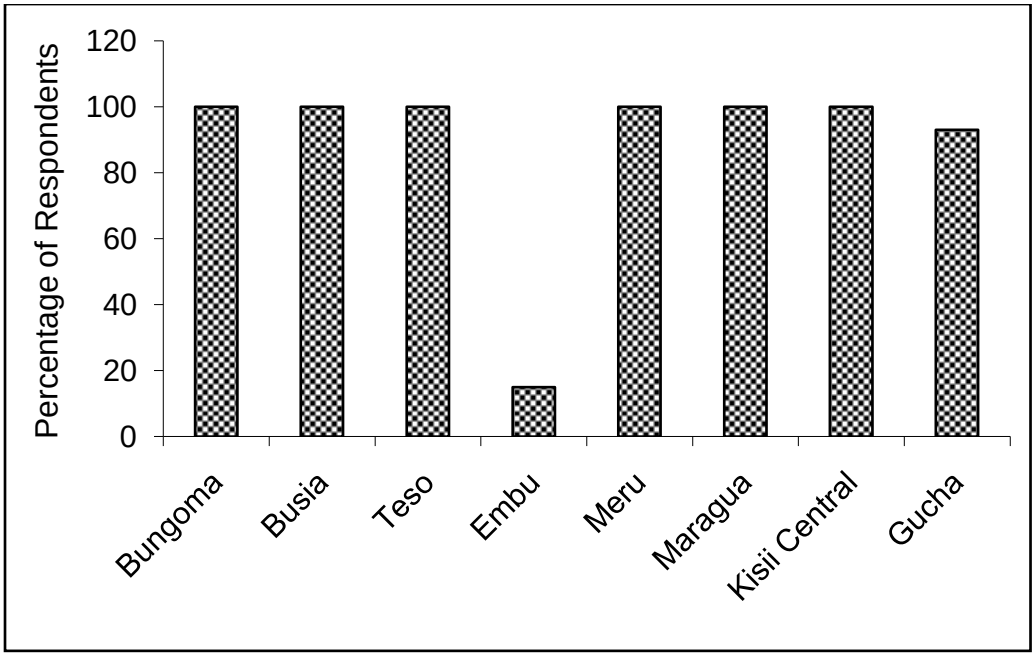


Fig. 5: Willingness of farmers to adopt new technologies to overcome constraints to banana production in six districts of Kenya

The interactions with farmers showed that banana is a major crop but faces several constraints. There is need to overcome BXW which is spreading fast but whose control measures are unknown to most farmers. Willingness of farmers to adopt new technologies appears to be influenced by need as well as socio-economic situation.

10.3.2 CONSUMERS

10.3.2.1 Consumer awareness of genetically modified crops

The survey results showed that there are differences in socio-economic characteristics that may influence awareness of biotechnology and genetically modified crops among consumers. Eighty six percent of all respondents are aware of biotechnology. Among males, 91% of those interviewed were aware compared to 75% recorded for female respondents. The sampled consumers were generally highly educated with up to 97.5 % having university qualification. In general, consumers in the higher socio-economic categories are more aware of biotechnology and GMOs.

Table 6: Consumers' awareness of biotechnology and genetic modifications by demographic characteristics

Variable	Category	Percent
Awareness		85
Gender	Male	75
	Female	91
Education	Informal	-
	Primary	-
	Secondary	55
	College	93
	University	98
Occupation	Professionals	100
	Industry	100
	Clergy	94
	Students	100
	Other sources	56
Information sufficiency	Sufficient	12
	Insufficient	88
Consumed GMOs	Food	35
	Drugs	1
	Vaccines	1
	Other	1

Among the well known GMOs include Bt Corn cited by 28.8% of the respondents, Bt cotton (8.9%) and transgenic potatoes (8.9%) (See Fig. 6.) Other biotechnology products that are familiar to consumers include TC bananas (14.9%) and seedless oranges (6.2%). Crops such as wheat, golden rice, canola and other biotechnology products amounted to 32.3%

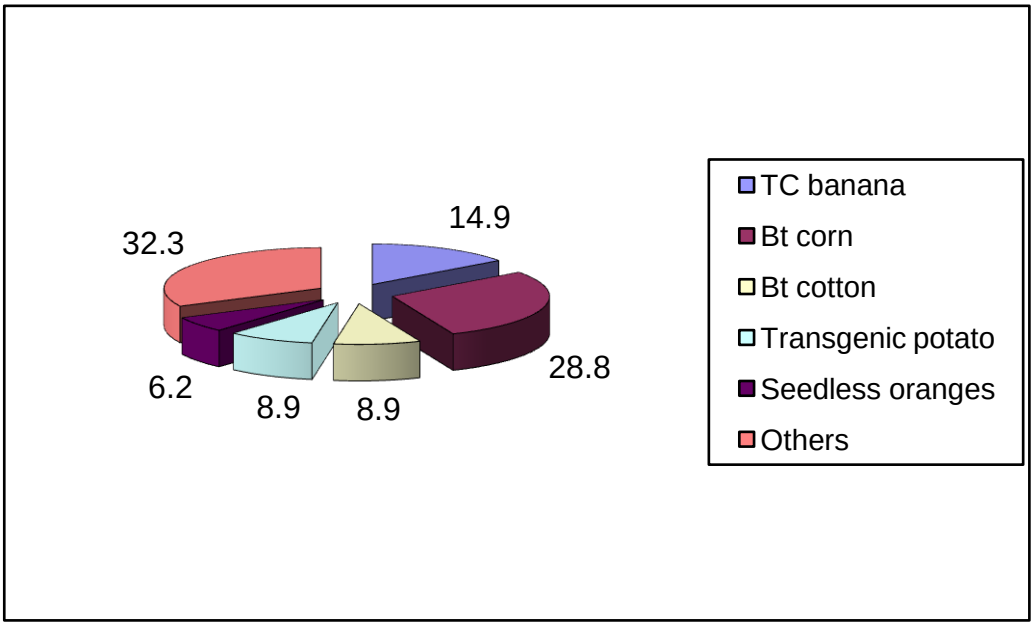


Fig. 6: Tissue culture and GM crops cited by consumers (expressed as a percentage of the respondents).

10.3.3 INDUSTRY AND TRADERS

Awareness of biotechnology and GMOs was highest in the category of industry and traders. All males (100%) interviewed were aware compared to 88% for women (Table 7). Respondents in this category tended to know a lot about biotechnology and GMOs (50%) and were well educated with a minimum of secondary education. Those who believed they consumed GMOs were more at 63% and mostly acquired from local market (33%). However, imported GMOs are also significant at 25%. Most companies (67%) have encouraging policies and believe that the future of biotechnology is bright.

Table 7: Industry and traders' awareness of biotechnology and GMOs by socio-economic characteristics

Variable	Category	Percentage
Gender	Male	100
	Female	88
Education	Secondary	17
	College	25
	University	58

Extent of awareness	A lot A little None	50 33 17
Sources of information	Radio Leaflets Media Other	- 29 29 42
Consumption		63
Acquisition	Local market Imported Other N/A	33 25 8 34
Company policy on GMOs	Encourage Discourage Other	67 8 25
Outlook	Bright Very bright Uncertain	42 25 33

SECTION VII

11.0 CONCLUSIONS AND RECOMMENDATIONS

- It is an acknowledged fact that banana bacterial wilt is a serious threat to banana production in the Great Lakes region.

- Lack of resistant germplasm has necessitated the search for viable means of achieving resistance such as genetic transformation.

- Given that the countries in Great Lakes region of Africa have already embraced biotechnology, we support the current initiative by AATF towards development of transgenic banana that is resistant to BBW.

- Our survey showed that there is already public acceptance for GM crops if this can help alleviate some of the problems currently being experienced by the farmers in the region.

- Given the success of the TC banana project, it is envisaged that farmers and consumers in Kenya and the Great Lakes region as a whole will also accept genetically transformed banana. What is important to the various stakeholders are the gains the new technology is likely to bring. If the benefits outweigh the risks then the technology is likely to be accepted.

- We also agree with Qaim's, (1999) conclusions that the banana TC project had opened up avenues for quick introduction of other biotechnologies that are most promising, especially for resource-poor farmers.

- As a team that participated in preparing this risk assessment report we recommend that the proposed transformation for resistance against BBW should be undertaken provided requisite information on Biosafety assessment is generated and supplied to regulatory authority as appropriate during the course of development and deployment of GM banana. The GM banana is likely to bring more benefits to the farmers just like the TC bananas are already doing.

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APPENDICES

Appendix 1: Time Schedule for Professional Personnel

Position	Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Days
Geneticist/Project Director/ Quality Management	Assure project direction and quality/Data collection/ Report compilation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	25
Environmentalist	Data collection and analysis, reports			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
Agronomist	Data collection and analysis, reports					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	21

Appendix 2: Team Composition and Task Assignments

Technical Staff

Name	Position	Task
Dr. Nelson Amugune	Project Director/Quality Management/Geneticist	Liaison with client and overall coordination of team/Data collection/Overall report compilation
Prof. J.I. Kinyamario	Environmentalist	Data collection and analysis, Report writing
Dr. W. Kimenju	Agronomist	Data collection and analysis, Report writing

APPENDIX 3: A summary of the information required to submit a proposal for the field release of transgenic plants

(This provides the basic information for risk assessment and should be in table form to be filled by Notifiers intending to release a GMO food/feed into the environment).

General information

Name and address of the organization wishing to release transgenic plants, including the names and qualifications of the personnel responsible should be supplied. It is considered important that the staff and the institution responsible for carrying out the release have a sufficiently high level of expertise and experience to carry out the proposed release of transgenic plants and to be responsible for any field containment

Information about the DNA donor organism, the recipient plant species and the transgenic plant

Characteristics of the transgene donor organism(s) and of the recipient plant species, including:

- scientific name and taxonomic details
- geographic distribution
- potential for genetic exchange with other organisms
- genetic stability
- pathogenicity
- toxicity
- allergenicity

Characteristics of the gene vector used to introduce the transgene(s) into the recipient plant species, principally:

- the nature and source of the vector
- properties of the DNA sequences present in the vector

Characteristics of the transgenic plant including:

- a description of the DNA sequences and the methods used to prepare and insert the introduced DNA
- the extent to which the introduced sequences are limited to the DNA required to perform the intended function(s) in the transgenic plant
- a description of the transgenic plant
- a description of how the genotype and phenotype of the transgenic plant differ from the plant it was derived from
- stability and level of expression of the transgene(s)
- allergenicity or toxicity of the transgenic plant products

Information about the conditions of the release and the receiving environment

A description of the proposed release, including:

- purpose of the release
- proposed planting date
- plot size
- number of transgenic plants
- agronomic methods
- methods of eliminating the transgenic plant material if found to be necessary

A description of the release site and the wider environment, including:

- geographical location
- proximity to humans
- local flora and fauna
- target and non-target ecosystems

Information about the interaction between the transgenic plants and the environment

Characteristics of the transgenic plant may affect its survival, multiplication and dissemination

A description of the interaction of the transgenic plant with its environment, including:

- relevant information obtained from earlier release studies on the likely environmental impact
- the possibility for gene transfer to other plants or to micro-organisms
- the possibility for dispersal of the transgenic plants themselves or their propagules
- methods used to verify genetic stability of the transgenic plants

An assessment of the potential environmental impact, including:

- the likelihood of excessive plant population increase
- the influence on non-target organisms

Information on monitoring, control and emergency response plans

a) A description of monitoring techniques, including:

- methods for identifying the transgenic plants
- methods for identifying the transgenes if transferred to other plants or organisms

b) A description of methods for controlling the site, including:

- minimising spread of transgenic plants
- methods to protect the site from intrusion

c) A description of methods of discarding waste plant material

d) A description of emergency plans to remove or destroy the transgenic plant material and to terminate and to terminate the experiment if it is considered necessary

The DNA donor organism, the recipient plant species and the transgenic plant:

It is essential to have information on the donor organism and the recipient plant species including:

- Information on the recipient species will establish a baseline against which to compare the transgenic plants.
- Knowledge of the donor species will highlight the kind of information required from the transgenic plant.
- If the donor is a plant pathogen, for example, this will raise questions in the risk assessment exercise about the possibility of recombination

between the integrated DNA from the pathogen and pathogens that may infect the transgenic plant subsequently.

Transformation vector:

Information is required on the DNA vector used during the transformation process to introduce the transgenes.

- Antibiotic resistance genes are generally used to facilitate the screening of transformed cells.
- Other DNA sequences may act as linking sequences with the vector or may provide other functions associated with the use of recombinant DNA methods
- With certain transformation systems carrier DNA is sometimes used to aid the transformation process. It is therefore necessary to know the nature of this DNA so that any consequences can be considered during the assessment process.

Transgenic plant:

It is important to give a description of the transgenic plant including

- molecular data on the inserted transgenes,
- the stability of expression,
- whether there is any change in allergenicity, toxicity and the capacity of the transgenic plant to persist in agricultural habitats or invade natural habitats.

It is essential here that the corresponding unmodified plant genotype is used as a control so that changes in plant phenotype caused by the transgenes can be measured.

The conditions of the release and the receiving environment*:*

- The risk to the environment requires qualitative judgments and, therefore, an essential part of the risk assessment philosophy is case by case analysis
- Providing information on the objectives of the release, its size and design and the agronomic treatments to be used are important both for

risk assessment of the particular release and for the longer term national and international learning process.

- Ecological information on the release site environment is also important. This should include a survey of plant species that might be growing in the vicinity of the release and information on what is known about the nature of pollen dissemination and the distances over which pollen can give successful pollination.
- The location and type of the anticipated target organisms must be specified. The target organisms are those which the transgenes are targeted to affect
- There should also be a consideration of whether the transgenic plant becomes a better or worse host and/or harmful to organisms that might be associated with the crop. The risk of harm to the environment includes harming non-target organisms.

The interaction between the transgenic plants and their environment:

In order to determine the impact of the transgenic plant on its environment it is important to describe changes in the transgenic plant that may change its invasiveness in wild habitats, its persistence in agricultural habitats or changes in its ability to propagate itself sexually or asexually.

It is also important to take a note of earlier studies with similar transgenic plants.

It is also necessary to determine the possibility of the transfer of transgene to the same or related plant species (wild and cultivated) or to micro-organisms and if this is possible what the consequences of that gene transfer might be.

Monitoring, control, waste treatment and the emergency response plans:

An important part of risk assessment is to determine the extent to which it is possible to monitor transgenes after the release and the efficiency with which it is possible to destroy plant material if it becomes necessary.

Efficient methods of identifying transgenic plants or transgenes in species they may have transferred to may be necessary. This may be by a visual marker (e.g. beta-*glucuronidase*), a selectable marker (e.g. antibiotic resistance) or by

molecular analysis e.g. PCR and Southern hybridization. It may also be appropriate to describe ways in which plant material can be destroyed at the end of the release experiment or if considered to be necessary during the course of the experiment.

Carrying out the risk assessment:

The aim of the risk analysis is to identify either changes to the experimental protocol or methods by which the GMO may be confined in order to minimize risk to the environment or human health.

It is difficult to put a value on the degree of risk. It is never possible to establish that releasing a transgenic plant will have no risk. The essential feature of risk assessment is to determine how the transgenes might alter risk compared with the non-transgenic crop; hence the starting point must be the use of the unmodified crop plant as a baseline against which to compare the effect of the inserted transgenes.

In cases where detailed scientific knowledge is not available, it is important to use the experience of conventional plant breeding to aid the risk assessment process. Plant breeding has been carried out for thousands of years and many of the genes being inserted by recombinant transformation fall into classes very similar to those manipulated by the conventional plant breeder. Some of the issues to be considered include the following:

What then, are the risks associated with the release of modified organisms?

Is it possible to confine the modified organism within the released site, and if it were to 'escape' would it pose a problem for the environment?

Could it, for example, survive or persist outside the managed 'site' within which it had been released?

There are differences in the potential of crop plants to transfer from the environment in which they are placed, and in their ability to establish feral populations. If this happened, would it matter?

Could the inserted genes be transferred to other plants of the same type or to wild relatives?

The risk assessment must take the host or parental plant as its starting point.

Is the host plant capable of surviving outside the normal agricultural environment?

Does it have relatives in the external or agricultural environment with which gene transfer is possible?

The modification must then be considered, both in terms of making any resulting transgenic plant more likely to survive and the safety of the gene product in the environment.

Field containment measures

There will be the opportunity for transgenic plants to be taken intentionally or inadvertently to other countries, including to those geographical areas that have a different spectrum of sexually compatible weed species.

A small scale risk assessment considers the distribution of sexually compatible species in the location of the release site. Large scale releases raise the question as to what geographical limits should be placed on the environment considered in the assessment scheme.

The experience of conventional plant breeding may make it more likely for resistant pests and pathogens to emerge. Other scale dependent questions regard the toxicity and allergenicity of the crop and the nature of the breakdown products of the transgenes when the plant decays.

It is important that the risk assessment leading to commercial release is thorough and open to scrutiny and considers all the evidence available from the small scale releases carried out with the same and similar transgenic plant material. There may be difficulties associated with large scale releases and some mitigating action might be considered for large scale releases.

There is now a wide range of plant promoters available for giving transgene expression in specific tissues in the plant. It may be desirable to restrict their expression of a particular protein to the parts of the plant where it is required. A

disease that attacks roots for instance may need an insecticide protein expressing only in roots.

When considering crop plants, it is not only their impact on the environment which needs to be considered, but also that they may be intended to be used for animal feed or human food. In addition, when the crop is out in the field, the farm-workers may come into contact with the plants or pollen at high concentrations. The possible toxic, allergenic, or carcinogenic properties of the modified plant have to be considered as part of the risk assessment.

APPENDIX 4: QUESTIONNAIRE ON PUBLIC PERCEPTION AND CONCERNS REGARDING THE USE OF BIOTECHNOLOGY PRODUCTS IN KENYA

ANNEX A: FARMERS

1. a) Name of Respondent ----------

b)Sex---------- male ⬭ Female ⬭

c) Province ---------- District ----------Location ----------Sub-location----

d) Level of Education

Primary ⬭ Secondary⬭ College ⬭ University ⬭ Informal ⬭

2. Occupation e.g.

- Farmer ⬭
- Extension Officer ⬭
- Other (specify) ⬭

3. a) What is the size of your farm? ------ acres.

b) What area is under banana? --------- acres.

4. Name five main crops including banana grown in your area and their ranking.

Crop	Ranking

5. What are the main banana types grown in your District?

Cooking ⬭ Dessert ⬭ Roasting ⬭ Beer ⬭

Ranking (Ist, 2nd , etc):Cooking ⬭ Dessert ⬭ Roasting ⬭ Beer ⬭

6. What are the main banana varieties grown and their ranking?

Variety	Ranking

7. What are the main sources of planting material (e.g. farmer's own, neighbour, TC, etc)?

Source	Ranking
1. Farmers own	
2. Neighbour	
3. TC Laboratory	
4. Research (KARI)	
5. Others (Specify)	

8. What are the current and historical uses of bananas in your area?

Current	Historical

9. What are the major constraints to banana production and their ranking?

Constraint	Ranking of the three most important
Soil Fertility	
Pests	
Diseases	
Drought	
Sourcing of planting materials	
Slow reproduction	
Others (specify)	

10. Name the major diseases affecting bananas in your district and their importance.

Disease	Importance (most important, important

11. What control measures have you adopted in banana bacterial wilt (*Xanthomonas* wilt) control? (Debudding, destruction of infected plants, sterilization of tools, crop rotation, etc.)

12. How effective are the control measures?

Effective ⬭ Slightly effective ⬭ Most effective ⬭

13. How fast is the banana wilt spreading in your district?

Absent ⬭ Slow ⬭ Moderate ⬭ Fast ⬭

14. Which banana varieties are affected by the disease?

Variety	Effect (Severe, moderate, slight)

15. In the opinion of the enumerator, assess the level of awareness of banana wilt among different stakeholders in the district.

Level of Awareness

Symptoms Low ⬭ Moderate ⬭ High ⬭

Spread Low ⬭ Moderate ⬭ High ⬭

Control Low ⬭ Moderate ⬭ High ⬭

16. Given choice to handle the challenges, would you accept a new technology that can control banana bacterial wilt? Yes ⬭ No ⬭

17. Have you heard about biotechnology products or Yes ⬭

genetic modifications?

No ◯

18. If yes, from which source?

Mass media ◯

Leaflets ◯

Radio ◯

TV ◯

Other (specify) ◯

19. Name some GMO crops/ products you are aware of

1) ----------
2) ----------
3) ----------

20. Given a choice between the existing conventional methods of disease control and biotechnology solutions, what would you choose?

Conventional ◯

Biotechnology ◯

ANNEX B: CONSUMERS

1. a) Name of Respondent _____

b) **Sex:** male ⬭ Female ⬭

c) Province _____ **District** _____**Location** _____**Sub-location**_____

d) Level of Education

Primary ⬭ Secondary ⬭ College⬭ University ⬭ Informal ⬭

2. Occupation e.g.

- Farmer ⬭
- Student ⬭
- Politician ⬭
- Clergy ⬭
- Professionals

  Scientists (researcher ⬭ Lecturer ⬭)
  Medical ⬭
  All others (e.g. teachers, lawyers, etc.) ⬭
- Industry ⬭
- Other (specify) ⬭

3. Under which organisation are you?

- Government ⬭
- Food industry/Flower company/Biotechnology company ⬭
- University/college ⬭
- Farmers' Union ⬭
- Environmental NGO. ⬭
- Member of an Expert Committee ⬭
- Consumer Association ⬭
- Research organisation ⬭

⬭

- Other (specify)

4. Have you heard about biotechnology products or genetic modifications?

Yes ◯

No ◯

5. If yes, from which source?

Mass media ◯

Leaflets ◯

Radio ◯

TV ◯

Other ◯

6. Name some GMO crops/ products you are aware of

1) ----------
2) ----------
3) ----------

7. Do you consume any of those products?

Yes ◯

No ◯

Not aware ◯

8. If yes, which products?

Food (specify) ◯

Drugs (specify) ◯

Vaccines (specify) ◯

Other (specify) ◯

9. How do you acquire them?

Imported ◯

From local market ◯

Other (specify) ◯

10. Do you have any interest in them?

Yes ◯

No ◯

11. If yes, which products?

Food (specify) ◯

Drugs (specify) ◯

Vaccines (specify) ◯

Other (specify) ◯

12. Is there enough information available on GMO products in Kenya?

Yes ◯

No ◯

13. If yes, where?

Government ◯

Research ◯

Organisation ◯

Internet ◯

News Papers ◯

Radio ◯

TV ◯

Other ◯

ANNEX C: INDUSTRY AND TRADERS

1. a) Name of Respondent _____

b) **Sex:** male ⬭ Female ⬭

c) Province _____**District** _____**Location** _____**Sub-location**_____

d) Level of Education

Primary ⬭ Secondary⬭College ⬭ University ⬭ Informal ⬭

2. If you are private, under which of this do you fall?

- Supermarket ⬭
- Industry ⬭
- Life science ⬭
- Flower Company ⬭
- Private laboratory ⬭
- Association ⬭
- Other ⬭

3. How long have you been in the business?

- 1 year ⬭
- 2 years ⬭
- 3 years ⬭
- 4 years ⬭
- 5 years ⬭
- 7 years ⬭

	Over 10 years	◯
4. Have you heard about biotechnology or genetic modifications?	Yes	◯
	No	◯
5. If yes, how much do you know about biotechnology and genetic modifications?	A lot	◯
	A little	◯
	Nothing at all	◯
6. If you know about biotechnology products and genetic modifications, do you consume any of those products?	Yes	◯
	No	◯
7. If yes, which products?	Food (specify)	◯
	Drugs (specify)	◯
	Vaccines (specify)	◯
	Other (specify)	◯
8. How do you acquire them?	Imported	◯
	From local market	◯
	Other. (specify)	◯
9. How do you inform the public about biotechnology products and genetic modifications?	Mass media	◯
	Leaflets/ pamphlets	◯
	Radio	◯
	Other	◯
10. Do you have any interest in genetic modifications (if you have little or nothing at all)?	Yes	◯
	No	◯
11. If yes, which ones?	Food (specify)	◯
		◯

Seeds (specify)

Other farm inputs (specify) ◯

Drugs (specify) ◯

Vaccines (specify) ◯

Other (specify) ◯

12. How do you acquire them?

Direct importation ◯

Technology transfer ◯

From other companies ◯

13. Is information available on GM and biotechnology products in Kenya?

Yes ◯

No ◯

14. If yes, where?

Government ◯

Research organisation ◯

Internet ◯

News Papers ◯

Radio ◯

TV ◯

Other ◯

15. How would you describe the future of biotechnology products and genetic modifications in Kenya?

Very bright ◯

Bright ◯

Uncertain ◯

16. What is your organisation's policy on biotechnology products and genetic modifications?

Encourage use ◯

Discourage the use ◯

◯

Other (specify)

APPENDIX 5: LISTS OF PARTICIPANTS WHO TOOK PART IN THE SURVEY

Appendix 5(a): Details of participants from industry and trade

	Name	Sex	Education	Institution/ Occupation
1.	Jotham Muriuki	Male	College	Industrial Area
2.	Reuben K. Soi	Male	University	KARI
3.	Harrison O. Lutta	Male	University	ABSF
4.	James K. Karanja	Male	University	KARI
5.	Rose Omamu	Female	University	KEPHIS
6.	Grace Mungai	Female	University	JKUAT

7. David N. Itigu l	Male	Secondary	Male Green Belt Movement
8. Churchill B. Kitau	Male	University	East African Seed Co.
9. Vincent K. Cheruiyot	Male	University	Kenya Bureau of Standards
10. Daniel Ojwang	Male	University	KIRDI
11. Stanley Atsali	Male	University	KIPI
12. Mwasi	Male	College	KIPI
13. Agronomist	Male	University	Mumias Sugar Co.
14. Andrew O. Nyakidi	Male	University	Mumias Sugar Co.
15. Agnes Waitherero	Female	Secondary	Trader
16. Eunice N. Mwangi	Female	Secondary	Trader
17. Eleanor Njoki	Female	College	Consumer Options
18. Mary Mwangi	Female	Secondary	Trader
19. James Njuguna	Male	College	Supermarket
20. Kate Achieng	Female	University	Flower Company
21. John Chege	Male	University	Genetic Technologies Ltd
22. Samuel Onyango	Male	University	Supermarket
23. Andrew Sifuna	Male	University	Life Science
24. Joseline Adeya	Female	University	Industry

Appendix 5(b): Details of participants (farmers) from Western province

Name	District	Division	Location	Sex	Educ.	Land size
1. Dimsus Simiyu	Bungoma	Malakisi	Lwandanyi	m	p	7
2. Wangusi Joseph	Bungoma	Malakisi	Lwandanyi	m	s	4
3. Dorothy Wabomba	Bungoma	Malakisi	Lwandanyi	f	p	2
4. Celina Tawai	Bungoma	Malakisi	Lwandanyi	f	c	7
5. Rosyline Matata	Bungoma	Malakisi	Lwandanyi	f	c	1
6. Joseph Mukhulu	Bungoma	Malakisi	Lwandanyi	m	s	1.5
7. Scholastic Nafula	Bungoma	Malakisi	Lwandanyi	f	p	5.5
8. Betty Wanyira	Bungoma	Malakisi	Lwandanyi	f	c	5
9. Gladys Barasa	Bungoma	Malakisi	Lwandanyi	f	p	1.5
10. Eunice Nelima	Bungoma	Malakisi	Lwandanyi	f	p	1.5
11. Henry Wanyonyi	Bungoma	Malakisi	Lwandanyi	m	s	2
12. Prisillah Omelo	Bungoma	Malakisi	Lwandanyi	f	l	5
13. Peter Tero	Bungoma	Webuye	Webuye	m	p	2
14. Peris Wanyama	Bungoma	Webuye	Webuye	f	c	2
15. Wilson Natse	Bungoma	Webuye	Webuye	m	s	3

16. Rosemary Khaemba		Bungoma	Webuye	Bokoli	f
	p 3				
17. Rose Lwiki		Bungoma	Webuye	Bokoli	f
	p 11				
18. Ben Walubengo		Bungoma	Webuye	Bokoli	m
	s 3				
19. Pius Waswa		Bungoma	Webuye	Matulo	m
	p 1				
20. Leonard Keya		Bungoma	Webuye	Matulo	m
	p 1.5				
21. Rhoda Munialo		Bungoma	Webuye	Matulo	f
	p 1				
22. Salome Simiyu		Bungoma	Webuye	Matulo	f
	s 1				
23. Anna Ebu		Busia	Matayos	Township	f
	l 3				
24. Bibiana Nyongesa		Busia	Matayos	Township	f
	l 9				
25. Crescentiae Mugeni		Busia	Matayos	W/ Bukhayo	f
	s 8				
26. Cleopas Oyombe		Busia	Matayos	W/ Bukhayo	m
	l 2				
27. Joseph Akombo		Busia	Matayos	W /Bukhayo	m
	l 0.5				
28. Tobias Antony		Busia	Matayos	W/ Bukhayo	m
	p 1.5				
29. Jane Marok		Busia	Butula	Lugulu	f
	l 6				
30. James Mugayi		Busia	Butula	Lugulu	m
	i 0.14				
31. James Sikuku		Busia	Butula	Lugulu	m
	p 2				
32. Margaret Maloba		Busia	Butula	Lugulu	f
	i 8				

33. Nathan Bisilwa p 1.25	Busia	Butula	Lugulu	m
34. Jentrix Juma p 1	Busia	Butula	Lugulu	f
35. Rosyline Auma p 2	Busia	Butula	Lugulu	f
36. Margaret Wandera p 0.5	Busia	Nambale	Bukhayo West	f
37. Martin Egesa I 4	Busia	Nambale	Bukhayo West	m
38. Nicasio Obore p 4	Busia	Nambale	Bukhayo West	m
39. Peter Wadeya p 2.5	Busia	Nambale	Bukhayo West	m
40. Silvanus Okumu p 4	Busia	Nambale	Bukhayo West	m
41. James Okumu I 3	Busia	Nambale	Bukhayo West	m
42. James Ndung'u I 2.5	Busia	Nambale	Town	m
43. Fridah Andanyi u 0.5	Busia	Nambale	Town	f
44. Morris Wambua u na	Teso	Amagoro	Amagoro	m
45. Musa Ochula s 4	Teso	Amagoro	Amoni	m
46. Samson Majoni p 4	Teso	Amagoro	Amoni	m
47. David Ekisa s 6	Teso	Amagoro	Koleu	m
48. Simon Ikobo i 1.25	Teso	Amukura	Aremit	m
49. Falton Wanzala i 2.5	Teso	Amukura	Aremit	m

50. Ali Mkwambo	Teso	Amukura	Aremit	m
p 1				
51. Mwanaisha Tiang	Teso	Amukura	Aremit	f
p 3				
52. Margaret Eshalayi	Teso	Amukura	Aremit	f
i 2				
53. Maurice Atelu	Teso	Amukura	Aremit	m
c 2.5				
54. Maciliano Ikokonyi	Teso	Amukura	Aremit	f
p 9				
55. Richard Terekwet	Teso	Amukura	Aremit	m
p 3				
56. Bramwel Ekatan	Teso	Amukura	Aremit	m
p 2				
57. Wycliff Ekutu	Teso	Amukura	Aremit	m
i 1				
58. Florence Teng'en	Teso	Amukura	Aremit	f
c 2.5				
59. Festo Manyala	Teso	Amukura	Aremit	m
p 1				
60. Getrude Amoit	Teso	Amukura	Aremit	f
p 3.5				
61. Braza Etiang	Teso	Amukura	Aremit	m
i 2				
62. Consolata Achieng	Teso	Amukura	Aremit	f
p 1				
63. Philip Baraza	Teso	Chakol	Alupe	m
s 3.5				
64. Robert Murega	Teso	Chakol	Asinge	m
c na				

Appendix 5(c): Details of participants (farmers) from Eastern province

Name	District	Division	Location	Sex	Educ.Land size
1. Alfred Njue	Embu	Central	Municipality	m	s 6
2. Boniface Mbogo	Embu	Central	Municipality	m	s 2
3. Mary Wanjiru	Embu	Central	Municipality	f	p 1
4. Lucy Wambeti	Embu	Central	Municipality	f	s 1
5. George Kariuki	Embu	Central	Municipality	m	s 2
6. Simon Njiru	Embu	Manyatta	Nginda	m	s 0.5
7. T. Nyaga	Embu	Manyatta	Nginda	m	c 4
8. Josephine Muthoni	Embu	Manyatta	Nginda	f	s 1
9. Jim Njeru	Embu	Manyatta	Nginda	m	s 1
10. Kellen Wambeti	Embu	Manyatta	Nginda	f	s 1
11. Johnson Njeru	Embu	Manyatta	Nginda	m	s 4
12. Naomi Njoki	Embu	Manyatta	Nginda	f	s 1
13. Lucy Mutitu	Embu	Manyatta	Nginda	f	s 3
14. Mrs. Njagi	Embu	Manyatta	Ngandori	f	p 6
15. Kinywa Mbui	Embu	Manyatta	Ngandori	m	s 2

16. E. Kathuraku	Embu	Manyatta	Central	m	c 5
17. Beatrice Kiura	Embu	Manyatta	Municipality	f	p 4
18. Njiru Mathembu	Embu	Manyatta	Municipality	m	p 2
19. S. Kinya	Embu	Manyatta	Ngandori	m	p 5
20. Nancy Njeru	Embu	Manyatta	Gaturi	f	s 1.5
21. Jeremia Mithika	Meru North	Ntonyiri	Akirango	m	p 1
22. Reuben Muturia	Meru North	Ntonyiri	Akirango	m	p 0.6
23. Esther Mwathimba	Meru North	Igembe N	Mburieru	f	i 0.1
24. Joseph Mungathia	Meru North	Igembe N	Mburieru	m	s 1.5
25. Martha Wanjohi	Meru North	Igembe N	Mburieru	f	p 1
26. John Birithu	Meru North	Igembe C	Kiengu	m	p 2.5
27. Geoffrey Mutua	Meru North	Igembe C	Kiengu	m	I 2
28. Zaccheus Kaituyu	Meru North	Igembe C	Kiengu	m	s 1
29. Eridah Kanaka	Meru North	Igembe C	Kiengu	f	s 3
30. Festus Munoru	Meru North	Igembe E	Kiengu	m	s 2
31. Isiaih Mwitha	Meru North	Igembe E	Kiengu	m	p 2
32. M’mwererea	Meru North	Igembe E	Kiengu	m	i 4

33. James Karimi	1	Meru North	Igembe E	Kiengu	f
34. Japhet Kithia	1.8	Meru North	Igembe E	Kiengu	m
35. Rose Kiludi	0.5	Meru North	Igembe E	Kiengu	f
36. Esther Nkirote	0.98	Meru North	Igembe E	Kiengu	f
37. Francis Mithika	6.8	Meru North	Igembe E	Kiengu	m
38. Yusuf Mung'atha	0.75	Meru North	Igembe E	Kiengu	m
39. Mackenzie Mureithi	2	Meru North	Igembe E	Kiengu	m
40. Fridah Kajuju	1	Meru North	Tigania East	-	f

Appendix 5(d): Details of participants (farmers) from Central province

Name	District	Division	Location	Sex	Education	Land size
1. Njuguna Benson	Maragua	Maragua	Nginda	m	s	3
2. Jackson Kamande	Maragua	Maragua	Nginda	m	p	4
3. James Gachanja	Maragua	Maragua	Ichagaki	m	s	0.5
4. Stephen Ndung'u	Maragua	Maragua	Ichagaki	m	s	1
5. Samuel Waweru	Maragua	Maragua	Ichagaki	m	c	2.7
6. Margaret Wairimu	Maragua	Maragua	Ichagaki	f	i	2
7. Edwin Kamau	Maragua	Maragua	Ichagaki	m	i	6
8. John B. Kingara	Maragua	Maragua	Ichagaki	m	p	2
9. Peter Waweru	Maragua	Maragua	Ichagaki	m	s	1
10. Mwangi Kamaguta	Maragua	Maragua	Ichagaki	m	p	2
11. Daniel Nduati	Maragua	Maragua	Ichagaki	m	s	10
12. Jackson Kamau	Maragua	Maragua	Ichagaki	m	c	2
13. James Maina	Maragua	Maragua	Ichagaki	m	p	1
14. Charles Kindia	Maragua	Maragua	Ichagaki	m	p	2.9
15. Samuel Njiva	Maragua	Maragua	Ichagaki	m	s	1

16. Ferisna Njoki 10	Maragua	Maragua	Ichagaki	f	i
17. Kimani 2	Maragua	Maragua	Ichagaki	m	u
18. John Mwangi 9	Maragua	Maragua	Ichagaki	m	p
19. Francis Njogu 12.6	Maragua	Maragua	Ichagaki	m	s
20. Laban Kagiki 1.4	Maragua	Maragua	Ichagaki	m	p
21. Francis Ngugi 0.5	Maragua	Maragua	Ichagaki	m	p

Appendix 5(e): Details of participants (farmers) from Nyanza Province

Name	District	Division	Location	Sex	Education	Land size
1. Dorcas Oside	Kisii Central	Kiamokama	Bogitaa	f	p	5
2. Alexina Monyina	Kisii Central	Kiamokama	Bogitaa	f	p	4
3. Betty Osugo	Kisii Central	Kiamokama	Bogitaa	f	c	5
4. Richard Omwono	Kisii Central	Masimba	Bomokora	m	s	6
5. Edward Omwono	Kisii Central	Masimba	Bomokora	m	c	1.5
6. John Koros	Kisii Ce	Masimba	Bomokora	m	s	3
7. Janeth Moragwa	Kisii Central	Masimba	Bomokora	f	s	10
8. Peter Ayora	Kisii Ce	Ibacho	Bonyanchaire	m	s	1
9. Paul John	Kisii Ce	Ibacho	Bonyanchaire	m	s	3
10. Charles Oriere	Kisii Ce	Ibacho	Bonyanchaire	m	s	4
11. Aloys Obiri	Kisii Ce	Ibacho	Bogiakumu	m	p	2
12. Jared Ayora	Kisii Ce	Ibacho	Bogiakumu	m	p	5
13. Thomas Okiki	Kisii Ce	Ibacho	Bogiakumu	m	p	2
14. Sanuel Mogaka	Kisii Ce	Keumbu	Bomariba	m	s	3
15. Patrice Omboto	Kisii Ce	Keumbu	Bomariba	m	i	5

16. Agnes Kerubos	4	Kisii Ce	Keumbu	Bomariba	f
17. Dominic Ogaros	5	Gucha	Nyacheki	Central	m
18. Evans Ororup	1	Gucha	Nyacheki	Boikang'a	m
19. Michael Ombogoc	2	Gucha	Nyacheki	Boikang'a	m
20. Raphael Oundus	3	Gucha	Nyamache	Botabora	m
21. Susan Nyanchokip	5	Gucha	Nyamache	Boige	f
22. Joyce Matangap	10	Gucha	Nyamache	Nyaramba	f
23. Christine Mayakap	5	Gucha	Nyamache	Bosaga	f
24. Lydiah Nyamachis	4	Gucha	Kenyenya	Central	f
25. John Atangas	4	Gucha	Kenyenya	Nyataro	m
26. Dominic Oinoc	5	Gucha	Tabaka	Bosinange	m
27. Amos Ngagwangap	4	Gucha	Tabaka	Bosinange	m
28. Hellen Biyakis	5	Gucha	Tabaka	Bosinange	f
29. Zebedeu Bitutus	1.7	Gucha	Tabaka	Bosinange	m
30. Nicholus Okebes	4	Gucha	Tabaka	Bosinange	m