Role of Biotechnology and Transgenics in bananas (*Musa* spp.) in Africa

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Abstract

In the more developed countries, genetically engineered crops contribute greatly to agricultural productivity and sustainability. Over the last few years, the largest growth in the adoption of genetically engineered crops has been in developing countries and this trend is expected to continue. The multinational life sciences companies have been leading the way, but they are focusing primarily on a few crop/trait combinations that have high commercial value and occupy large international markets. Because of the costs and complexity of the issues related to crop biotechnology, many crops and traits of importance to subsistence and resource poor farmers around the world have been overlooked. The Agricultural Biotechnology Support Project (ABSPII), a Cornell University-led and USAID funded consortium of public and private sector institutions, provides support for scientists, regulators, extension workers, farmers and the general public in developing countries to make informed decisions about agricultural biotechnology. When possible, ABSPII creates public-private partnerships to help leverage public funds to help absorb development costs and provide broader distribution channels. Since 2005, ABSPII has been working with the National Agricultural Research Organization in Uganda to establish safe and cost effective programs for the development and commercialization of East African highland bananas (*Musa* spp.), genetically engineered for black sigatoka and nematode resistance. This paper gives a brief description of the work that has been done to date and discuss the ABSPII based strategy that has been adopted to develop and deliver genetically engineered crops for developing countries.

INTRODUCTION

In many developed countries, genetically engineered crops already contribute greatly to agricultural productivity and sustainability. Over the last few years, the largest growth in the adoption of genetically engineered crops has been in developing countries and this trend is expected to continue (James, 2007). Multinational life sciences companies have been leading the way, but they are focusing primarily on a few crop/trait combinations that have high commercial value and occupy large international markets. Because of high development costs, nominal returns on investment and complexity of the issues related to crop biotechnology, many crops and traits of importance to subsistence and resource poor farmers have been overlooked.

The Agricultural Biotechnology Support Project (ABSPII), a Cornell University led and USAID funded consortium of public and private sector institutions, provides support for scientists, regulators, extension workers, farmers and the general public in developing countries to make informed decisions about agricultural biotechnology (Gregory et al., 2008). Where demand exists, ABSPII works with local institutions to establish safe and cost effective programs for the development and commercialization of genetically engineered crops that otherwise would not be developed. When possible, ABSPII creates public-private partnerships to help leverage both public and private funding sources to help absorb development costs and provide broader distribution channels. ABSPII currently is working in India, Bangladesh, Philippines and Uganda to develop products with the intention of reducing poverty and alleviating hunger. In Uganda, ABSPII is supporting a project focused on the improvement of the East African highland banana (EAHB) for disease and pest resistance using the most modern technologies available at the moment.

IMPROVEMENT OF EAST AFRICAN HIGHLAND BANANA IN UGANDA

EAHB, known locally as matooke, is one of the most important food and cash crops in Uganda, as measured by production output, acreage, consumption levels and priority ranking by stakeholders (Kikulwe et al., 2008). During the last thirty years, there has been a drastic decline in production in the traditional banana growing areas of the Central region and production has shifted to Southwestern Uganda. As determined by Uganda's National Agricultural Research Organization (NARO), the most serious constraints to banana production are black sigatoka (syn. black leaf streak virus, caused by *Mycosphaerella fijiensis*), nematode (*Radopholus similes*) and weevil (*Cosmopolites sordidus* Germar) infestation. Black sigatoka alone can reduce yields by 30–50% and the disease affects all traditional banana cultivars in West and Central Africa and most of the widely grown cultivars in Eastern Africa. There are several nematode species that attack banana in Uganda and cumulative yield losses attributed to nematodes are estimated to be up to 51% by the fourth crop year. Most of the EAHB cultivars are also quite susceptible to attack by banana weevils, a serious stem borer pest. Under severe weevil infestation, crop losses of up to 100% have been reported. Bacterial wilt (caused by *Xanthomonas campestris* pv. *musacearum*), a relatively new disease to the region, is emerging as a serious constraint as well. Bacterial wilt infection results in complete loss of crop, rather than just lowered productivity. Considerable losses of EAHB and other dessert banana types are also caused by the *Banana bunchy top virus*, banana streak viruses and Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *cubense* 'tropical race 4').

Conventional banana breeding relies on crossing highly sterile cultivated triploids with wild diploid relatives followed by extensive backcrossing. Depending on the trait of interest, this can be a long, laborious and often unsuccessful process. Genetic engineering offers the possibility of moving desirable traits into acceptable banana germplasm in a more precise and timely manner. Genetic transformation of EAHBs is now possible in several cultivars and technologies have been developed that may offer resistance to pests and diseases, improved nutrition via bio-fortification, delayed ripening or prolonged shelf life, yield enhancement and overall production enhancement.

Since 2000, the Government of Uganda has contributed funds (as subscription to the Consultative Group of International Agricultural Research (CGIAR)) to the International Network for the Improvement of Banana and Plantain (INIBAP, now part of Bioversity International) to coordinate a program to develop national capacity in banana biotechnology and technology transfer to address three major constraints to banana production in Uganda, including black sigatoka, nematodes and weevils through the use of biotechnology. The INIBAP/Bioversity led consortium was put together with the purpose of assisting the banana research program of Uganda's NARO to transform EAHBs, discover suitable genes and evaluate transformed lines. The USAID funded ABSPII banana project has successfully built upon the existing platform to accelerate Uganda's biotechnology product development and commercialization strategies in genetically engineered EAHBs. It is hoped that these capacities can be used to solve similar problems in other crops of national interest.

ASBPII has worked closely with the management and researchers at NARO to restructure their research strategy and to prioritize the set of activities to be carried out by the new consortium of partners involved. The project partner institutions included the Kathelieke Universiteit Leuven (KUL), the University of Leeds, Cornell University and Uganda's NARO. ABSPII provided technical oversight and backstopping to oversee the design of a state-of-the-art banana tissue culture laboratory, the building of a research team with the skills necessary to develop a reproducible transformation system, and the capacity to develop and test genetically engineered EAHBs relevant to local need. More recently, ABSPII facilitated the construction of a containment greenhouse facility and confined field trial site at the National Agricultural Research Laboratories Institute (NARLI) at Kawanda, Uganda.

Initial priority setting exercises established that technology was available that may be useful for improving EAHBs for resistance to black sigatoka and nematode infestation. Although banana weevil is a significant banana pest, no insecticidal protein technology was available for consideration. For black sigatoka resistance, antifungal chitinase genes from rice have been transformed into the 'Gros Michel' cultivar (AAA genome) at KUL by a Ugandan scientist who has since returned to NARO and is continuing his work on developing improved gene transformation systems and applications for the improvement of EAHBs. For nematode resistance, EAHBs have been transformed with a maize cystatin gene at the laboratory in Uganda. Several independent events have been generated and will soon be tested under greenhouse and contained field trial conditions.

TRANSFORMATION AND REGENERATION IMPROVEMENTS

During the first phase of the ABSPII project, the team at NARO successfully developed embryogenic cell suspensions (ECS) from several EAHB cultivars and two dessert cultivars. An *Agrobacterium* mediated gene transformation protocol was established to genetically transform the EAHB ECS. Successful transformation was achieved for three EAHB cultivars ('Nakinyika', 'Nakasabira' and 'Mpologoma'; AAA-EA genome) as well as for the desert banana cultivar 'Sukali Ndiizi' (AAB genome). To test transgenic plants safely and follow the Uganda national guidelines for genetically modified organisms (GMO) containment, a biosafety greenhouse was constructed with the support of the ABSPII project, which was completed in May 2007.

BLACK SIGATOAKA RESISTANCE

Black sigatoka is a leaf spot disease of banana that begins with black spots, which eventually expand to cover the whole leaf, leading to premature drying and significant yield losses. Wind, rain, old planting material and irrigation water spread black sigatoka from plant to plant. All EAHB cultivars are susceptible to black sigatoka and yield reductions often reach 30–50%. A few black sigatoka resistant hybrids, introduced by FHIA, have been released to Ugandan farmers, but none of the hybrids meet the culinary preferences of local consumers. The disease is difficult and expensive to control. Commercial growers in wealthier countries use regular aerial fungicide spraying to control black sigatoka. This crop management practice is not feasible under current conditions in Uganda.

During his PhD training at KUL, Dr. Geofrey Arinaitwe generated transgenic plants of the banana cultivar 'Gros Michel' that carried rice chitinase genes (*rcc2* or *rcg3*) for resistance to black sigatoka. In August 2007, the genetically engineered banana plants were imported to Uganda from Belgium for the first field trial of genetically engineered crops in Uganda. After a hardening period, the plants were planted in a confined field trial at NARLI in November 2007 and are currently under evaluation. The field trial application and approval process in Uganda was a significant capacity building exercise for NARO. The process took nearly two years to complete but much was learned and it is anticipated that future application processes will be handled more effectively. It is also anticipated that new policies and legislation will soon be adopted in Uganda that will allow for the commercialization of genetically engineered crops.

NEMATODE RESISTANCE

Nematodes have been identified as a key pest of banana in Uganda. Yield losses commonly reach 30–50% due to nematode induced suppression of plant growth, growth of root rots that cause toppling in high winds and by predisposing the plants to weevil damage. Due to the high cost of nematicides and the lack of other cost effective control options, genetic engineering for nematode resistance may be the best pest control option for nematode infestation. The research team at NARLI has successfully generated transgenic EAHB, containing a maize cystatin gene known to confer nematode resistance in other crops. This was achieved using constructs designed and made by the University of Leeds partners. Plants have been regenerated in NARLI and are currently being evaluated for maize cystatin gene expression and stable gene integration. Results from these studies will be the basis for selecting a sub-set of lines that should advance to field trial. It is anticipated that durable nematode resistance will require expression of several resistance genes in the same plant. New transformation vectors are being designed and constructed that combine the rice cystatin gene with second generation resistance technologies in an effort to develop banana plants with more durable resistance against a wider range of the nematodes species that attack banana. A comprehensive product development strategy has been developed to quickly assess the best combination of gene technologies. Only the most promising events will be tested in the field for efficacy and biosafety studies.

BACTERIAL WILT RESISTANCE

The banana *Xanthomonas* wilt (BXW) is the most devastating disease of banana in Great Lakes region of East and Central Africa. The pathogen kills plants quickly and spreads rapidly over a large area in a short time, making the disease one of the most dreaded in banana. The disease affects almost all cultivars of commonly grown banana cultivars, including EAHBs. Economic impact of the disease is manifested as result of absolute yield loss or reduced bunch weights, and death of the mother plant and suckers that help in subsequent ratoon plant production cycles. Diseased fields cannot be replanted with banana due to soil borne inoculum of the pathogen. The symptoms of the disease are yellowing and wilting of leaves, unevenly and premature ripening of fruits. Finally all leaves wither and the plant rots.

The development of disease resistant banana cultivars remains a high priority, since farmers are reluctant to employ labor intensive disease control measures. Prospects of developing cultivars with resistance to BXW through conventional breeding are limited, as no source of germplasm exhibiting resistance against *Xanthomonas* has been identified. Transgenic technologies for banana may provide a timely and cost-effective alternative solution to the BXW pandemic.

Leena Tripathi's group of the International Institute for Tropical Agriculture (IITA), in collaboration with NARO, developed a new *Agrobacterium*-mediated transformation system, using intercalary meristematic tissue (Tripathi et al., 2008a). The system is working well for several different cultivars of EAHBs. This is the first report of a rapid and efficient protocol for EAHB using a cultivar independent transformation system and may thus provide an important tool for the genetic improvement of *Musa* species, including EAHB cultivars.

Using this new system, many different banana cultivars have been transformed with a vector that expresses the reporter gene beta-glucuronidase (*gus*A). Several lines have been generated that are positive for GUS activity. The resulting fully-rooted transgenic plants do not appear to be chimeras, since they can be stably propagated. GUS activity is observed uniformly throughout the plants including the germline cells of the meristem and PCR and southern blot data indicate stable integration of the genes into the genome. Research is underway to confirm the genetic stability of DNA transferred to bananas using this technology.

Dr. Tripathi's team, in collaboration with NARO, the African Agricultural Technology Foundation (AATF) and Academia Sinica, Taiwan, has also introduced gene encoding ferredoxin-like amphipathic protein (*pflp*) into EAHBs cultivars in an effort to develop Xanthomonas wilt resistant bananas. The ferredoxin-like amphipathic protein (*pflp*), isolated from *Capsicum annuum* is novel protein that can intensify the harpinPSS-mediated hypersensitive response and has conferred resistance against bacterial diseases in many other crops (Tripathi et al., 2008b).

Several transformed lines of banana cultivars 'Pisang Awak' (ABB genome), 'Mpologoma' and 'Nakitembe' (AAA-EA genome) have been generated, which are currently under screening for disease resistance in laboratory conditions. Plants shown to be transgenic for the *pflp* gene via PCR analysis are being tested by artificial inoculation to evaluate for BXW resistance under controlled laboratory conditions. Some promising lines show no or delayed symptoms of BXW infection. These lines will be further tested in pots in the containment facilities.

COMMUNICATION

For Ugandan stakeholders to make informed decisions related to the potential use of genetically modified products dependents, they must have access to high quality science based information. ABSPII's outreach activities in Uganda have focused on building early awareness and understanding of genetically engineered crops. A 'frequently asked questions' sheet has been developed to help policy makers and members of the banana research team answer inquiries from the media. This FAQ sheet will be further refined and made available to the public. The main objective of the communication effort is to minimize the impact of non-scientific misinformation that is commonly disseminated by those opposed to the development of modern technologies. Members of the banana research team have on many occasions hosted local, regional and international media to explain crop biotechnology actives and several media reports have been produced. Most notably, the research was featured on BBC Jimmy's Farm documentary in the United Kingdom in December 2008 and on BBC TV/radio world news in June 2008.

PRODUCT DEVELOPMENT PATHWAY

The product development and commercialization process will require multi-location trials, cultivar registration, release and dissemination, together with careful product awareness campaigns in which private public partnerships will be developed for plantlet development and delivery. Biosafety assessments of all genes, gene products and plant parts will need to be done before deregulation and commercial release of a product. ABSPII will work closely with NARO to facilitate public and private relationships with local banana seedling producers to make high quality GM bananas plants available to growers. Emphasis will be placed on training banana plantlet producers how to test for presence of a specific transgene and to produce disease and virus free material. Plantlet producers will also be trained to help them develop marketing strategies and distribution channels.

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