Bacterial Wilt-Resistant BANANA

Project Progress Report 2012

Improving Banana for Resistance against *Xanthomonas* Wilt in Sub-Saharan Africa

Message from the Project Coordinator

Bananas are among the most important food crops after maize, rice, wheat, and cassava. Annual production in the world is estimated at 130 million tonnes, nearly one-third of it being grown in Sub-Saharan Africa (SSA), where the crop provides more than 25 percent of the food energy requirements for over 100 million people. East Africa produces and consumes the most bananas in Africa, with Uganda being the world's second largest producer after India.

Banana farmers especially in Uganda are currently battling with the anguish of watching their fruit ripen prematurely, seeing their leaves wilt and then witness the inevitable death of the entire plant.

The Banana Xanthomonas Wilt (BXW) pathogen, the cause of this phenomenon, costs banana farmers millions of dollars in damages every year across East and Central Africa. The pathogen infects all varieties, including East African Highland Banana and the exotic types. The rapid spread of the disease has endangered the livelihoods of millions of farmers who rely on banana for staple food and cash.

There are presently no commercial chemicals, bio-control agents or resistant varieties that could control the spread of BXW. Even if a source of resistance is identified soon, developing a truly resistant banana through conventional breeding would be quite difficult and would take years, given the sterile nature and long gestation period of the crop.

Transgenic technologies that facilitate the transfer of useful genes across species have been shown to offer numerous advantages to avoid the natural delays and problems in breeding bananas. These technologies provide a cost-effective method to develop varieties resistant to BXW.

The novel green pepper proteins that give crops enhanced resistance against deadly pathogens can also provide effective control against other BXW-like bacterial diseases in other parts of the world. The mechanism of this resistance is such that it rapidly kills plant cells at the region of attempted invasion by the pathogen and forms a physical barrier to prevent further infection. In addition, it also activates the defences of surrounding and even distant uninfected parts of banana plants leading to a systemic acquired resistance.

The transgenic approach brings in more options of defence against the wilt but more significantly, it presents real optimism of saving livelihoods in Africa.

Dr Jacob H Mignouna
Project Coordinator

Background

Bananas and plantains are an important food source for over 100 million people in SSA. In the east African highlands and most of the Great Lakes region, bananas are a major staple food and a source of income for over 50 million smallholder farmers. East Africa produces about 20 percent of the world output. However, many living and non-living factors greatly reduce productivity for banana cultivated under traditional African farming systems. For instance, as a result of the BXW banana disease, a 50 percent decline in household incomes from banana sales and a corresponding increase in banana prices were observed during 2001 and 2004 in Uganda. Other costs associated with BXW include labour for cutting down and disposing of infected plants, de-budding the male flowers and disinfecting cutting tools. These cultural disease control methods currently in use have been useful in confining the spread of the disease and the on-going work to develop disease resistant material will enhance what has been achieved so far.

A banana bacterial wilt infected plant – the spread of the disease has affected the livelihood of thousands of farmers who rely on the crop as staple food and for income generation

The BXW disease caused by *Xanthomonas campestris* pv. *Musacearum*, was first reported about 44 years ago in Ethiopia on *Ensete* (a native plant to Ethiopia) which is closely related to banana and then it was also discovered on banana. Outside of Ethiopia, BXW was first identified in Uganda in 2001 and subsequently reported in the Democratic Republic of Congo, Rwanda, Tanzania and Kenya. Its rapid spread has endangered the livelihoods of thousands of farmers who rely on banana as a staple food and for income generation. The disease is very destructive, infecting all banana varieties. Prospects of developing varieties with resistance to bacterial wilt through conventional breeding are limited, as no source of germplasm exhibiting resistance against BXW has been identified. Transgenic technologies for banana may provide a timely alternative solution to the BXW pandemic.

Project overview

The plant ferredoxin-like protein (*pflp)* gene and the hypersensitivity response assisting protein (*hrap)* gene isolated from sweet pepper by Dr Teng-Yung Feng of Academia Sinica in Taiwan has been identified as a possible solution for developing a transgenic banana resistant to BXW.

AATF brokered access to this gene from Academia Sinica and is working with partners - the International Institute of Tropical Agriculture (IITA) and the National Agricultural Research Organisation (NARO) in Uganda - to develop a *Xanthomonas* wilt-resistant banana from East African preferred germplasm.

The outputs expected from the project include proof that either the *hrap* or *pflp* gene confers resistance to BXW and the development and testing of transgenic lines with *hrap* and *pflp* genes. It is also expected that there will be additional cultivars of banana/plantain transformed in Uganda and Kenya.

It is expected that smallholder farmers in SSA will have access to adapted high yielding bananas from east African highland germplasm with resistance to BXW by around 2017.

Smallholder farmers in Sub-Saharan Africa are expected to have access to the adapted high yielding bananas with resistance to Xanthomonas wilt disease by 2017

Project management

During the reporting period, AATF filed an application for the patent registration of the new *pflp* gene at the African Regional Intellectual Property Organisation office to protect the new *pflp* gene in Uganda and Kenya.

A project review and planning meeting was held in July 2012 to take stock of progress and redefine roles and responsibilities of partners in project implementation. During the meeting, scientists working on the project reported that they have generated several lines of the banana cultivar *Sukali ndiizi* popularly known as *apple banana* that have shown resistance to the disease. The lines are undergoing testing under confined field trials (CFTs) at the Kawanda research station in Uganda. The meeting was also attended by participants drawn from various research institutes involved in banana research in Kenya, Tanzania, Rwanda, Burundi and Uganda.

Partners who attended the review and planning meeting in July 2012 visiting the confined field trial site in Kawanda, Uganda

Product development progress

Sixty-five transgenic plants and non-transgenic mother plants have been evaluated for BXW resistance in CFTs in Uganda and assessed for disease symptom development. All transgenic lines tested have significantly higher ($P \leq 0.05$) resistance in comparison to control non-transgenic plants. However, 12 lines (7 lines with *Hrap* gene and 5 lines with *Pflp* gene) have shown resistance to BXW and are being evaluated for second ratoon plants. The mother plants were harvested in December 2011. The bunch weight and size of transgenic lines are similar to non-transgenic plants. The best 10 lines will be tested further with more replicates in a second trial through the support of the United States Agency for International Development's Agricultural Biotechnology Support Programme.

Results from the trials demonstrated that constitutive expression of the sweet pepper *Hrap* or *Pflp* gene in banana resulted in enhanced resistance to *Xanthomonas* wilt. More transgenic lines with additional farmer preferred cultivars will be generated by IITA at the Biosciences east and central Africa (BecA) hub in Kenya.

The confined field trial site in Uganda where both transgenic and non-transgenic mother plants were evaluated for banana bacterial wilt resistance

About 300 transgenic plantlets of plantain cultivar *Gonja manjaya* were obtained and validated for presence of transgene by polymerase chain reaction (PCR) analysis. These lines are now under multiplication for evaluation for disease resistance under glasshouse.

Also more than 100 transgenic lines of *Sukali Ndiizi* and 200 lines of *Gonja manjaya* with stacked *Hrap* and *Pflp* have been generated and validated by PCR. Three replicates of about 150 transgenic lines with stacked genes were evaluated for BXW resistance by artificial inoculation of potted plants in screen house. More than 30 lines with stacked genes showed resistance. Evaluation of additional lines is on-going and disease indexing of plants with stacked and single genes will be used to calculate significant differences.

Confined field trial compliance

Investigations on the efficacy of transgenic banana have continued at NARO in Uganda since October 2010 in compliance with requirements pertinent for transgenic plants under confined field conditions. A compliance audit mission carried out by AATF at the CFT site in July 2012 did not reveal any incidences of compliance infraction. In addition, a CFT compliance management training for CFT site staff was carried out in November 2012 in Uganda.

Some of the partners during the CFT compliance management training in November 2012 in Uganda

Communications and outreach

A draft communications plan for 2012/2013 was developed to support information dissemination, awareness building and dialogue exchange with policy makers, farmers and stakeholders. A story on progress of the project was posted on the AATF website http://www.aatf-africa.org/Developing-bacterial-wilt-resistant-banana.

Capacity enhancement

In the area of capacity enhancement for the Project, two scientists continued with their studies. Muwonge Abubaker who is in his fourth year of training is writing a Ph.D. Thesis "Enhancing resistance against Xanthomonas campestrisis pv. Musacearum in bananas by co-expressing *pflp* and *hrap* genes" at the University of Pretoria, South Africa. Kenneth Mburu, a Kenyan Ph.D. student, is currently training in banana tissue culture and transformation.

The future

As the Project progresses into 2013, the various activities towards providing smallholder farmers with BXW resistant varieties will continue. More lines will be generated for additional cultivars with vector *hrap*, *pflp* and *hrap/pflp* in Kenya and Uganda.

In addition, 300 transgenic lines of the *Gonja manjaya* species developed by IITA-at the BecA hub will be maintained and evaluated for resistance to BXW in glasshouse.

All the transgenic lines developed in the lab are with vectors whereby the gene expression was under regulatory control of the CaMV35S constitutive promoter. The project will test whether or not using stronger promoters like maize ubiquitin enhances disease resistance. More plasmid vectors will be constructed using the maize ubiquitin promoter and independent transgenic lines with each construct will be generated.

Transgenic lines will be developed with additional cultivars, including preferred plantain and dessert bananas in East and Central Africa. Transgenic lines with stacked genes will then be tested for enhanced and durable resistance and the best lines selected for CFT.

A CFT is expected to be conducted in Kenya to evaluate transgenic lines for BXW resistance in 2014.

Project Partners

- African Agricultural Technology Foundation (AATF)
- Academia Sinica
- International Institute of Tropical Agriculture (IITA)
- National Agricultural Research Organisation of Uganda (NARO)
- Public and private tissue culture laboratories in the Great Lakes region of Africa including Burundi, Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda

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