Weevil resistant sweetpotato through biotechnology

SASHA
Sweetpotato Action for Security and Health in Africa

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Weevils damage about a quarter of the sweetpotato harvest in Uganda and induce the accumulation of toxic compounds in the healthy-looking parts of damaged storage roots. We have introduced new genes that produce anti-weevil proteins in the sweetpotato storage root through biotechnology. We may have found few with some resistance to weevils. In parallel, we are testing a new strategy to weaken specific genes of the weevils to block their development. These two strategies will eventually be combined into widely-cultivated sweetpotato varieties in SSA.

Storage roots infected by *Cylas puncticollis*

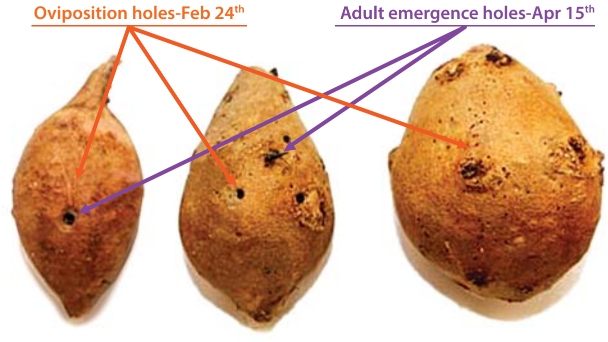

Fig 1. Weevil resistance test showing transgenic event b undamaged

Weevils threaten food security of the poorest

A farmer survey conducted in Uganda revealed that weevils are responsible for 28% of crop losses every year. Losses can be up to 90% during dry periods, which can be quite devastating. Weevils can affect not only food security, but also sweetpotato production, marketability, healthiness, and sustainability, especially in areas experiencing longer dry periods. With climate change predictions for Sub-Saharan Africa (SSA) foreseeing an expanding dry season, the threat and impact of weevils may increase further. Adapting conventional integrated pest management practices among smallholder farmers is difficult due to the challenges controlling field sanitation in small-scale production systems. Extensive efforts to develop weevil-resistant sweetpotato through conventional breeding have not yet succeeded. As a result, there is currently little farmers can do when weevils infest their fields, other than quickly harvest and salvage what is left of their crop.

What do we want to achieve?

The aim of this project is to develop weevil-resistant sweetpotato varieties through breeding and biotechnology. *Bacillus thuringiensis (Bt)* is a soil bacterium that is well-known for its insecticidal activity. Synthetic genes that produce the proteins active against the two weevil species attacking the sweetpotato can be developed and introduced into the plant to confer pest resistance. Because the storage root has low protein content, a non-protein-based approach might be needed at the same time. Therefore, RNA interference (RNAi) targeting sweetpotato weevil essential genes can be combined with the insecticidal proteins to provide stable resistance to sweetpotato weevils. This *Bt* – RNAi combined gene technology has already been used successfully to increase resistance to rootworm in maize. In addition, health benefits may be expected because farmers will not consume partially damaged roots containing toxic compounds as they do currently under severe food shortage.

How are we working with partners?

Research on the identification of insecticidal proteins from *Bt* (Cry proteins) has taken place in the US at the Auburn University and at the National Crops Resources Research Institute (NaCRRI) in Uganda. Genetic transformation of sweetpotato was first developed at the CIP biotechnology lab in Peru, later at Makerere University and NaCRRI in Uganda, then at BecA and Kenyatta University in Kenya, and lately at the Donald Danforth Plant Science Center in the USA. A confined field trial has been conducted at the

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Sweetpotato for Profit and Health Initiative
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Partners include:

Biosciences for East and Central Africa (BecA), Kenya
National Crops Resources Research Institute (NaCRRI), Uganda
Kenyatta University, Kenya
Donald Danforth Plant Science Center, USA
University of Ghent, Belgium
University of Valencia, Spain
University of Puerto Rico Mayagüez, USA

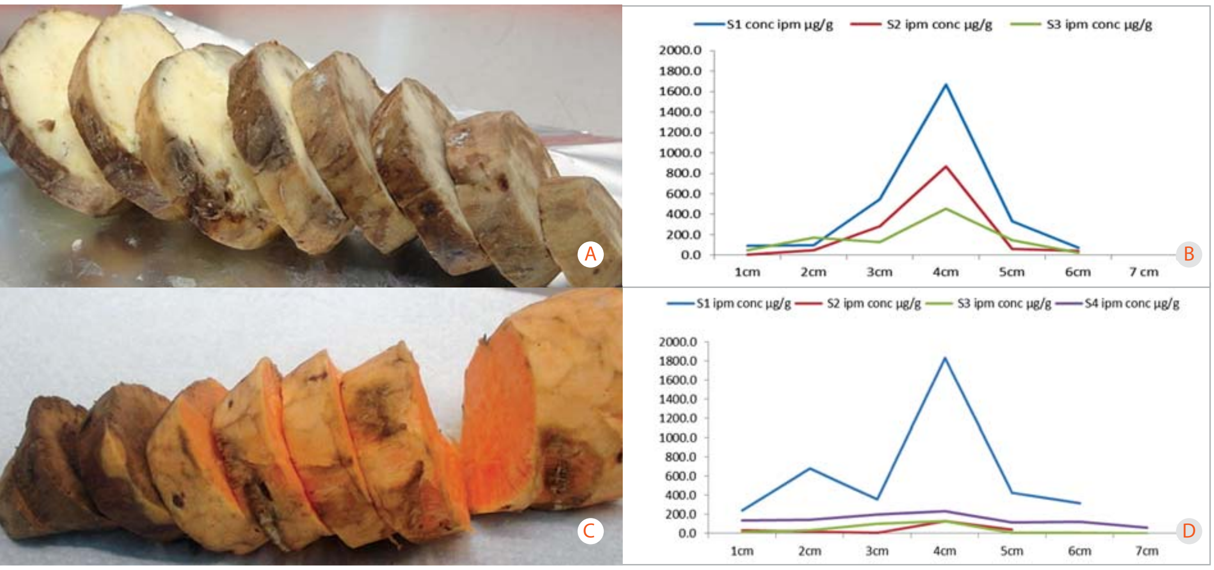

Fig 2. Accumulation of ipomeamarone in healthy parts of fungal infested sweetpotato storage roots

University of Puerto Rico Mayaguez. The University of Valencia elucidates the mode of action of the Cry proteins and Ghent University builds mainly biosafety capacities and is developing the RNAi strategy. The project is targeting Uganda and, if successful, other SSA countries.

What have we achieved so far?

We have introduced synthetic *cry* genes that produce proteins with activity against sweetpotato weevils into various sweetpotato varieties, including some grown in SSA. A total of 117 transgenic plants with *cry* genes were produced and many of them were tested for resistance against weevils. So far, most of them did not show activity against weevils but two storage roots did not have any visible weevil damage, and two did not produce adults (Fig. 1). These cases will be reconfirmed with new fresh storage roots. New *cry* gene constructs, designed for higher cry gene expression, were used to produce almost 500 transgenic plants in three laboratories. Those with the highest Cry protein levels are now under evaluation for resistance against weevils, with results expected by the end of 2015 for the *Bt* strategy.
The RNAi strategy is taking shape with the confirmation that when any of three specific genes of the weevil are inhibited by RNAi, the larvae die. Accordingly, five hairpin gene constructs were designed to target essential genes of the weevils. Soon genetic transformation of the variety 'Jonathan' will be initiated.
Research on the quantification of ipomeamarone produced in storage roots in response to fungal infection was completed and published in 2015.

This study highlights a potential health threat to farmers in SSA who consume the undamaged parts of infected storage roots as meaningful levels of toxin were found in the undamaged parts (Fig. 2). Additional research is needed, however, to quantify the presence of ipomeamarone under varying conditions of storage, cooking method, varietal type, etc.
Capacity strengthening continues. Our third African PhD candidate completed this year. One remaining PhD candidate at Ghent University is halfway through.

What are the next steps?

The testing of resistance to weevils of *Bt* sweetpotatoes has been slow due to a number of unfavorable factors: the time-consuming protocol for genetic transformation of this crop, the need to produce storage roots in pots in contained facilities, and complications transferring plant material from Peru to the USA and to African countries. After six years, we have now reached the final step of testing resistance to weevils using storage roots produced in the greenhouse. In parallel, the RNAi strategy looks promising and therefore will be combined with the previous one provided any of the transgenic plants with *Bt* reconfirms to have some level of resistance. Finally, we firmly believe that the Cry protein expression, possibly combined with RNAi, will confer weevil resistance --which remains the single most important threat on sweetpotato food availability to the poor in many SSA countries.

Fig 3. The evil sweetpotato weevil

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