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 new proteins and small RNA against weevils in the sweetpotato storage root through biotechnology. We may have found few plants with some resistance to weevils. In parallel, we continue producing more plants with small RNA to weaken specific genes of the weevils to block their development. These two strategies may eventually be combined into widely-cultivated sweetpotato varieties in SSA.

Fig 1. Storage roots of transgenic events at five weeks after oviposition from 10 female adults. Left event DDPSC 109-114 1 showing multiple tunnels and alive larvae; Right Bt DDPSC 109 115 showing small tunnel with no larvae (credit D. Ndege)

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 and build capacity in Biotechnology research in SSA. *Bacillus thuringiensis* (*Bt*) is a soil bacterium that is well-known for its insecticidal activity. Synthetic *Bt* genes that produce the proteins active against the two weevil species attacking the sweetpotato were introduced into the plant but failed to confer complete pest resistance. Because the storage root has low protein content, a non-protein-based approach (RNA interference - RNAi) is targeting essential genes of the weevils and can be combined in the future with the insecticidal proteins. 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

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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

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 University of Puerto Rico Mayaguez. The University of Valencia elucidated 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.

Fig 2. (Left) Normal pupal development. (Right) Dead pupae after 14 days treated with dsRNA against Ribosomal protein s13e (G19) (credit K. Prentice)

What have we achieved so far?

We have introduced synthetic *cry* genes that produce Cry proteins with activity against sweetpotato weevils into various sweetpotato varieties, including some grown in SSA. A first group of 63 transgenic events was tested for resistance against weevils. Bio-assays using storage roots infested by oviposition from female adults resulted in 6 transgenic events with apparent differences with the non-transgenic materials. Two of them (Jonathan with *cry7A* and *cry3C* genes) did not produce adults and are pending confirmation of resistance. A more recent series of transgenic sweetpotatoes bearing high-constitutive-expression *cry* genes was produced. The second group of 69 transgenic events was tested and 6 of them (two Imby with cry3C and 4 PI531122 with either one (2), or two (2) cry genes) were found with apparent differences with the non-transgenic materials. Hence, out of a total of 132 transgenic events tested, we have identified 12 with apparent differences with the non-transgenic materials (Fig 1). This percentage of around 10% is within those observed for engineered trait relying on transgene expression level. However, we have observed significant variation in infection efficiency from assay to assay and we cannot rule out that these 12 events were simply poorly infected. Therefore, we will reconfirm these results with new fresh batch of storage roots.

An RNAi strategy was developed to complement the Bt strategy. Our partners at the University of Ghent have identified 3 target genes that have given good mortality results for both weevil species in both soaking and artificial diets: proteasome 20 kD subunit, ribosomal protein S13e and *snf7* genes (Fig 2). Five hairpin gene constructs were designed based on *Prot20kd* and *snf7* from *Cylas puncticollis* (Cp) and *C. brunneus* (Cb) in single and double combinations. Genetic transformation is on-going using the best variety for *Agrobacterium tumefaciens*-mediated transformation (Jonathan) via somatic embryogenesis methods which were optimized previously as well as the faster but less efficient method of organogenesis. 400 explants (meristems) and 4,800 leaves with petioles have been agro-infected with the first gene construct received (*snf7* of *C. puncticollis*). Over 20 transgenic events have been isolated and more transformation experiments are on their way. These will be screened for high expressers of small RNA and then storage roots produced and tested at the BecA facilities.

So far three African studies have obtained their doctorates under this program, with one more still at the research stage.

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 seven years, we have now reached the final step of testing and reconfirming 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.

CONTACT
Marc Ghislain (CIP)
m.ghislain@cgiar.org