Weevil resistant sweetpotato through biotechnology

SASHA Sweetpotato Action for Security and Health in Africa

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Biotechnology has been applied to introduce synthetic genes that produce proteins with activity against sweetpotato weevils. The analyses of almost 100 transgenic events did not result in the identification of high accumulation of the Cry protein in the storage root. New cry genes designed for higher accumulation have been introduced in sweetpotato varieties and will be tested by the end of the year. In parallel, dsRNA against several weevil essential genes were shown to kill larvae when micro-injected. These results profile the new resistance strategy based on Bt and RNAi technology.

Sweetpotato weevil larva in damanged root (credit Anon)

What is the problem?

A farmer survey conducted in Uganda revealed that weevils are responsible for 28% of crop losses. As sweetpotato is at times the only food available, this can be quite devastating. A recent study has also highlighted potential health threat when the undamaged parts of the roots are consumed due to high accumulation of the toxin ipomeamarone. The impacts of weevils can affect not only food security, but also sweetpotato production, marketability, 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 does not seem promising because of the great difficulty associated with controlling field sanitation in small-scale subsistence production systems. Extensive efforts to develop weevil-resistant sweetpotato through conventional breeding methods have failed so far in spite of considerable investment for decades. As a result, there is currently little farmers can do when weevils infest their fields, other than to quickly try to 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 biotechnology. *Bacillus thuringiensis* (Bt) is a soil bacterium that is well-known for its insecticidal activity mediated by crystal (Cry) proteins. Synthetic genes that produce the proteins active against specific insect pests can be developed and introduced into the target plant to confer pest resistance. This so-called Bt technology has been used successfully to increase resistance to bollworm in cotton and rootworm or stem borer in maize. There have been highly significant results indicating reduced pesticide use, increased biodiversity, and increased yields in those crops including non-Bt crops cultivated in their vicinity. Farmers, including small-scale producers, have been the primary beneficiaries of growing Bt crops. In the case of Bt sweetpotato, farmers will be able to harvest only what is needed while the rest of the crop will remain stored it in the soil and protected from weevil devastation. 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.

Who are we working with?

Research on the identification of insecticidal proteins from Bt (Cry proteins) has taken place in the USA at the University of Auburn and in Uganda at the National Crop Resources Research Institute

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C. puncticollis **larvae at 5 days after injection** (Bottom). **Control larvae injected with milliQ water (Top).** In the treatments, third-instar larvae were injected with 200 ng of dsRNA against ribosomal protein S13e.

(NaCRRI). The genetic engineering technology has taken place first at CIP biotech lab ABL in Peru and later at the University of Makerere and NaCRRI both in Uganda, and BecA and Kenyatta University in Kenya, and recently at the Donald Danforth Plant Science Center in USA. A confined field trial has been conducted at the University of Puerto Rico Mayaguez (USA). In Europe, the University of Valencia (Spain) has worked on mode of action of the Cry proteins. Finally, the University of Ghent (Belgium) has contributed to capacity building for African partners and in partnership with Venganza ltd (USA) to the development of RNAi technology. The project is targeting primarily sweetpotato production in Uganda and possibly Kenya.

What have we achieved so far?

Between 2004 and 2007, with Rockefeller Foundation funding, we identified four Cry proteins exhibiting useful weevil toxicity. Soon after, weevil-resistant (WR) genes were developed using genetic information from the sweetpotato crop itself in order to make 3 sweetpotato-like WR genes. Since 2009, the Bill & Melinda Gates Foundation and USAID have funded the next developments. The WR genes were introduced into sweetpotato varieties but the low levels of Cry protein detected in the storage roots could not control the weevils. Hence, a new design and combination of cry genes was conceived. Research at the University of Valencia demonstrated that Cry7Aa1 and Cry3Ca1 share the same insect gut receptor. Accordingly, new WR genes were developed using the cry3Ca1 and the ET33 and ET34 genes expressed independently. Transgenic events are being produced and expected to be tested for control of weevils by the end of 2014. Since sweetpotato tuberous roots are naturally poor in protein, we are pursuing an RNAi technology. The University of Ghent has shown that micro-injected dsRNA against several essential genes kill *C. puncticolis* larvae. Oral feeding studies with the best dsRNA need to confirm these promising results before we embark on a genetic transformation combining Bt and RNAi technologies.

What's next?

The testing of resistance to weevils has been slow due to a number of unfavorable factors: the time-consuming protocol for genetic transformation of this crop, the need to produce tuberous roots in contained facilities, the transfer of plant material from Peru to the US and to African countries. The transgenic events did not accumulate enough Cry protein to control weevil larvae. Therefore, the new gene constructs expected to accumulate more Cry protein may provide some control of both African weevil species. However, we firmly believe that the Cry protein expression combined with RNAi will confer resistance to weevils which remain the single most important threat on sweetpotato food availability to the poor in many Sub Saharan African countries.

Transgenic sweetpotato plants in BecA greenhouse in Kenya (credit M. Ghislain)

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