Weevil resistant sweetpotato through biotechnology

SASHA Sweetpotato Action for Security and Health in Africa

AUG 2013

Biotechnology has been applied to introduce synthetic genes that produce proteins with activity against the weevils. Out of about 30 transgenic events, one with the Cry3Ca1 gene displayed reduced infection and damages by weevils in a confined field trial in Puerto Rico. New gene constructs expected to result in enhanced accumulation of weevil-active proteins are now being used to transform sweetpotato varieties. As a complementary strategy, an RNAi strategy started with transcriptome analysis to identify weevil target genes. As this brief goes to press, first toxicity assays are currently underway on weevil larvae.

Damage caused by weevils on sweetpotato roots (credit Robert Mwanga)

1. Weevils threaten food security of the poorest

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. The impact 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 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.

2. Gene technologies to protect sweetpotato

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 specific insect pests can be developed and introduced into the target plant to confer pest resistance. For example, this so-called *Bt* technology has been used successfully to increase resistance to bollworm in cotton and rootworm or stem borer in maize. The result has been reduced pesticide use and increased yields in those crops. 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 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.

3. A successful international partnership

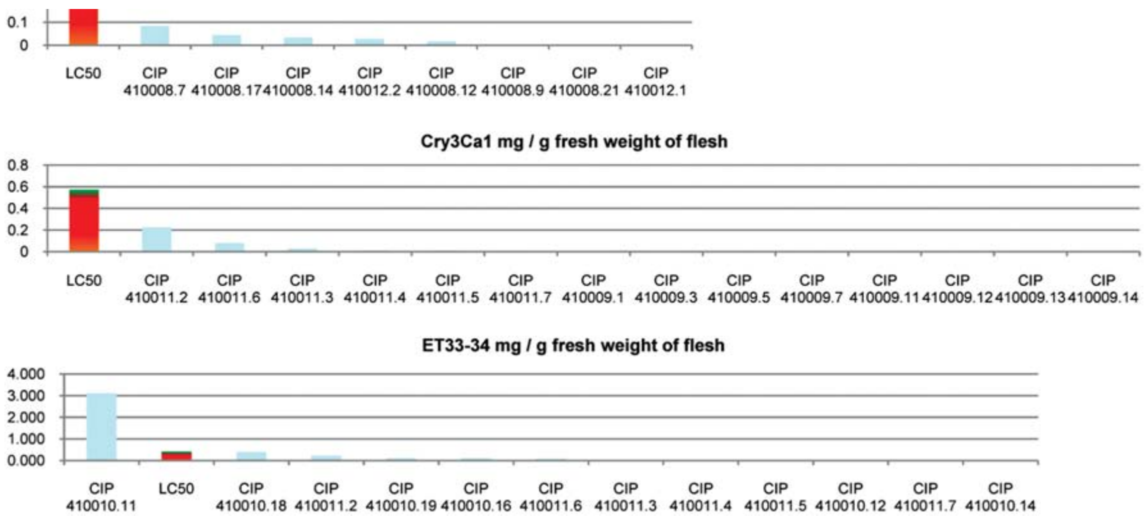
Research on the identification of insecticidal proteins from *Bt* (Cry proteins) has taken place at the University of Auburn (USA) and at National Crop Resources Research Institute NaCRRI in Uganda. The genetic engineering technology has taken place first at CIP biotech lab ABL in Peru and later at the University of Makerere and NaCRRI both in Uganda, and BecA and Kenyatta University in Kenya. A confined field trial has been conducted at the University of Puerto Rico Mayaguez (USA). Two European laboratories, the University of Valencia

CENTRO INTERNACIONAL DE LA PAPA • CIP

A member of the CGIAR Consortium

Partners include:

- National Crops Resources Research Institute (NaCRRI) and Kawanda Research Station (KARS) of NARO, [Uganda] for developing and testing resistance to weevils in sweetpotato plants, including a confined field trial
- Biosciences east and central Africa (BecA), Kenyatta University (KU)-[Kenya] for developing and testing resistance to weevils in sweetpotato plants
- University of Puerto Rico Mayagüez, Auburn University-[USA] for testing resistance to weevils in sweetpotato plants including a confined field trial
- Donald Danforth Plant Science Center- [USA] for guidance and oversight in the development of regulatory dossier
- University of Ghent- [Belgium] for capacity building of African scientists and developing communication products



Quantification of Cry proteins of transgenic events versus LC50 (red)

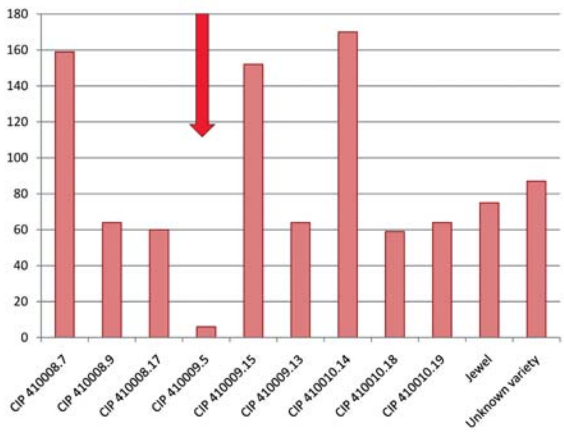
and the University of Ghent, have worked on mode of action of the Cry proteins and capacity building respectively. Risk assessment is led by the Donald Danforth Plant Science Center in US. The project is targeting primarily sweetpotato production in Uganda and possibly Kenya.

4. Achievements

Between 2004 and 2007 with Rockefeller Foundation funding, we identified three distinct 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 failed to control the weevils. Our research indicates that the level of accumulation of the Cry proteins is the likely cause of lack of efficacy because their quantities were lower than the lethal dose killing 50% (LC_{50}) larvae in artificial diet assay. Although one event displayed accumulation above LC_{50}, it failed to provide control of the weevil. This has raised doubts about the functional activity of the fusion protein ET33-34. A confined field trial conducted in Puerto Rico confirmed the absence of weevil control but one transgenic events expressing Cry3Ca1 displayed sub-lethal activity. Research on the mechanism of toxicity at the University of Valencia indicates that Cry7Aa1 and Cry3Ca1 may share the same insect gut receptor. Accordingly, new Cry genes were developed to enhance accumulation and many more transgenic events will be tested in the coming two years. Because of the results observed so far, we will focus on the *Cry3Ca1* and the *ET33* and *ET34* genes expressed independently. Since sweetpotato tuberous roots are naturally poor in protein, we have added a complementary strategy based on RNAi. Together with Venganza Ltd, the University of Ghent has made good progress in identifying candidate genes from the two African weevils by transcriptome analyses. Bioassays using African weevils are currently underway.

5. Future outlook

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 USA and to African countries. Therefore, an extension of this project by several months will be desirable to complete the evaluation of the existing and the new transgenic events for efficacy against both African weevil species. Finally, we firmly believe that the Cry protein expression possibly 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.



Emerging adults from 17 sweetpotato roots per transgenic event

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