Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda

Research Application Summary

Enhancing sweetpotato resistance to African weevils (*Cylas puncticollis* and *Cylas brunneus*) through transgenic breeding

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Abstract

Sweetpotato production is greatly constrained by two weevil species; *Cylas puncticollis* and *C. brunneus* that cause yield losses of up to 80%. The use of resistant sweetpotato cultivars is the most sustainable strategy to control the weevils. However, little success has been realized in developing resistant cultivars. Genetic engineering is a more viable option that offers a means to introduce resistant genes into sweetpotato. In this study the transgenic variety Jewel events with *cry7Aa1*, *cry3Ca1*, *ET33-34*, *cry7Aa1+cry3Ca1* and *cry7Aa1+ ET33-34* will be used as source of resistance in transgenic breeding. The progenies will be selected for the presence of the Bt protein product and evaluated for weevil resistance and agronomic performance. The best performing progenies will be further used in the sweetpotato breeding programme to enhance resistance to the African weevils.

Key words: Bt protein, genetic engineering, *Ipomea batatas*, weevil resistance

Résumé

La production des patates douces est considérablement menacée par deux espèces de charançon: *Cylas puncticollis* et *C. brunneus*. Ces espèces causent des pertes de rendement allant jusqu'à 80%. L'utilisation des variétés résistantes de patate douce est la stratégie la plus durable pour surveiller ces charançons. Cependant, peu de succès ont été réalisés dans le développement des variétés résistantes. Le génie génétique est une option plus viable qui offre un moyen d'introduire les gènes résistants dans la patate douce. Dans cette étude, les résultats de Jewel sur la variété transgénique avec *cry7Aa1*, *cry3Ca1*, *ET33-34*, *cry7Aa1+cry3Ca1 et cry7Aa1+ ET33-34* seront employés comme source de résistance dans la reproduction transgénique. Les progénitures seront choisies en fonction de la présence du produit protéinique Bt et évaluées en fonction de la résistance face au charançon et de la performance agronomique. Les meilleures progénitures en

performance seront encore employées dans le programme de reproduction des patates douces pour améliorer la résistance contre les charançons africains.

Mots clés: Protéine Bt, génétique, *Ipomea batatas* , résistance des charançons

Background

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is an important crop in East Africa where it is grown as a staple food (Stevenson *et al*., 2009). For some farmers, the crop also supplements family income, and, thus, strategies to reduce losses to pests and diseases provide opportunities to enhance food security and improve livelihoods. In Africa, in general, the sweetpotato weevils *Cylas puncticollis* Boheman and *C. brunneus* Fabricius are the major production constraints, whereas in America and Asia *C. formicarius* is the major pest (Andrade *et al*., 2009). In areas where weevils are endemic, damage is also recorded during the growth season and production losses reach up to 60-100%. In Uganda, the two species may cause yield losses of up to 80% (Smit *et al*., 2001). Even low levels of infestation can reduce root quality and marketable yield because the plants produce unpalatable terpenoids in response to weevil feeding (Stathers *et al*., 2003).

Sweetpotato weevils attack stems, crowns and roots which render them difficult to control and their cryptic habit reduces the effectiveness of control by chemical or biological insecticides and parasites (Smit *et al*., 2001). Despite years of intensive research, varieties with resistance to *C. puncticollis* and *C. brunneus* are not available despite the progress in finding weevil resistant components in some varieties (Stevenson *et al*., 2009). Sweetpotato producers in Sub-Saharan Africa are mostly small-scale, resource-poor farmers growing the crop all year round. Hence, management strategies that have been proven successful in Cuba or pilot sites are unsuitable to African farmers. In contrast, resistant varieties would be easily adopted resulting in boosting production dramatically with a positive impact on people's livelihoods. In this context, the prospect of control by introducing weevil resistance genes through genetic engineering using *Bacillus thuringiensis* (Bt) technology is an attractive one (Cattaneo *et al*., 2006).

Literature Summary

The use of Bt crops has resulted in a significant reduction in insecticide use, yield increase, increased grower profitability, and increased diversity of non-target insects (Cattaneo *et al*.,

2006). This makes exploring the Bt option particularly attractive given the lack of progress with conventional breeding. Delta-endotoxins from *Bacillus thuringiensis* (Bt) have been used against *Cylas puncticollis* and *C. brunneus*, and *C. formicarius* (Andrade *et al*., 2009). An *in vitro* insect feeding assay indicated that diet formulations including specific Bt proteins were highly toxic to the three weevil species (Moar *et al*., 2007). Following these results, five sweetpotato WR gene constructs have been developed using chemically synthesized DNA sequences for the coding and polyA regions and two promoters known for driving high expression levels in sweetpotato roots (Kreuze *et al*., 2009). The difficulties to transform and regenerate local sweetpotato genotypes with the WR gene constructs have limited the introduction of the novel trait (Moar *et al*., 2007). Consequently, the Bt genes can be introduced into local cultivars through conventional breeding, using a genetically engineered sweetpotato as a source germplasm for the WR genes. This method referred to as transgenic breeding has been used in potato (Johnson *et al*., 2003) and maize. Nevertheless, very little has been done to use this technology for improving sweetpotato production in Uganda and worldwide.

Study Description

The study is being carried out at the National Crops Resources Research Institute (NaCRRI), Uganda. The research is being conducted in three phases. The variety Jewel events different Bt genes (*cry7Aa1*, *cry3Ca1*, *ET33-34*, *cry7Aa1+cry3Ca1* and *cry7Aa1+ ET33-34*) will be evaluated for weevil resistance. The assay technique to be used is an adaptation of one previously described by Stathers *et al.* (2003). The tests will be conducted in a randomized complete block experimental design. Those events which perform best would later be crossed to the local varieties in a North Carolina mating design II under confined environment approved for the work with transgenic material. At flowering, hand pollination will be done. The genetically engineered Jewel genotypes will be used as female parents. The obtained F_1 hybrid will undergo PCR analysis to determine the presence of the Bt genes. Tripple antibody sandwich (TAS)-ELISA will be done to detect the expression levels of the Bt protein in the F_1 sweetpotato genotypes. The transgenic material will be tested in the field according to the Uganda national biosafety regulation for confined field trial. The transgenic F_1 hybrids, non transgenic F_1 hybrids and reference varieties (checks) are planted in a randomized block

design with 3 replications and planted on mounts at 1m X 1m spacing (10 plants of each genotype per replication).

Research Application

This research is expected to have a transgenic breeding methodology established using Bt genes for weevil resistance. It is anticipated that weevil resistant genotypes with good quality and yield traits will be developed which can be released as WR varieties or used by other breeding progammes.

Acknowledgement

This study is jointly funded by RUFORUM and the International Potato Centre.

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