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Research Application Summary

Efficient regeneration and transformation systems for improving resistance to weevils in Ugandan sweetpotato cultivars

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Abstract

Sweetpotato is cultivated worldwide as a valuable source of food. Weevils cause 60-100% loss of productivity. The present study aims at genetically transforming selected Ugandan sweetpotato cultivars with genes that confer resistance to weevils. A tissue culture method is being optimised to be coupled with *Agrobacterium* transformation technique for regeneration of transgenic sweetpotato. Preliminary results show ability to form embryogenic callus in six tested cultivars. Magabali cultivar has shown best response of 72% embryogenic callus. Plant genotype, plant organ and concentration of growth hormone in media have shown significant effect on somatic embryogenesis and could affect plant regeneration efficiency.

Key words: *Agrobacterium* transformation, *Ipomoea batatas*, plant regeneration, weevil resistance

Résumé

La patate douce est cultivée dans le monde entier comme source adéquate de nourriture. Les charançons causent la perte de productivité pouvant s'étendre de 60% à 100%. La présente étude vise à transformer génétiquement les cultivars sélectionnés de patate douce ougandais avec les gènes qui transmettent la résistance aux charançons. Une méthode de culture de tissu est optimisée pour être couplée à la technique de transformation d'*Agrobacterium* pour la régénération de la patate douce transgénique. Les résultats préliminaires montrent la capacité de former le calus embryogénique dans six cultivars examinés. Le cultivar de Magabali a montré la meilleure réponse du calus embryogénique de 72%. Le génotype de la plante, l'organe végétal et la concentration de l'hormone de croissance dans les médias ont montré l'effet significatif sur l'embryogenèse somatique et pourraient affecter l'efficacité de régénération de la plante.

Mots clés: Transformation d'*Agrobacterium*, *Ipomoea batatas*, régénération de la plante, résistance de charançon

Background

Sweetpotato yields in Sub-Saharan Africa are as low as 5 t/ha, compared with the world's average of 15 t/ha (Luo *et al.*, 2006). The low yield is mainly due to pests such as weevils (Stathers *et al.*, 2003). The biology of sweetpotato presents many challenges to the application of crop improvement techniques, in particular for weevil resistance. The application of biotechnology tools to complement present breeding efforts has great potential. This study aims at genetically transforming selected Ugandan sweetpotato cultivars with genes that could confer resistance to weevils.

Literature Summary

Genetic transformation allows insertion and expression of specific genes, from related or unrelated species, into the genome of a target organism (Shimada *et al.*, 2006). This advantage extends to use of synthesized genes that have more similarities in codon usage to the genes of the target plant.

An efficient and reliable transformation and regeneration system is a pre-requisite for successful application of biotechnological approaches in plants (Aloufa, 2002). Sweetpotato lacks such a system.Existing regeneration and transformation protocols are still highly genotype-dependent (Santa-Maria and Pecota, 2009) which represents a serious limitation due to high genetic diversity of existing varieties. This genotype dependency has been ascribed to anthocyanins and polyphenolic compounds (Islam *et al.*, 2002). The protocol by Song *et al.* (2004) with minor modifications is being tested in this study for its application in regenerating plants from Ugandan sweetpotato cultivars through somatic embryogenesis. Song *et al.* (2004) used 4-fluorophenoxyacetic acid 4-FA for callus induction on Linsmaier and Skoog (LS) medium.

Study Description

The plant regeneration study is being conducted in the tissue culture laboratory at Makerere University Agricultural Research Institute-Kabanyolo (MUARIK) and the National Crops Resources Research Institute (NaCRRI) in Uganda. In the study, leaf and petiole explants of *in vitro* plants and different combinations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylamino Purine (BAP) are being used. Factors like plant genotype, plant organ used, auxin and cytokinin concentration in Murashige and Skoog (MS) media are being investigated for their effect on somatic embryogenesis and subsequent plant regeneration.

The methods for *in vitro* regeneration being optimized at NaCRRI is an initial step towards developing an efficient

transformation strategy for selected Ugandan sweetpotato cultivars for improved resistance to weevils.

Research Application

Eight of the selected 20 Ugandan sweetpotato cultivars have successfully been established *in vitro*. These cultivars were first bio-indexed (Fig. 1A) before preparation of *in vitro* cultures that are used as source of petiole and leaf explants (Fig. 1B) for callus induction. All eight cultivars tested have shown ability to form callus. However the calli produced were both embryogenic and non-embryogenic (Table 1). Embryogenic calli were identified by their bright yellow colour (Fig. 2) and soft texture (Song *et al.*, 2004). The texture of explants-derived calli is considered an important factor in sweetpotato plant regeneration. The hard and compact nature of Type II calli probably contributes to the difficulty in regeneration (Liu and Cantliffe, 1984).

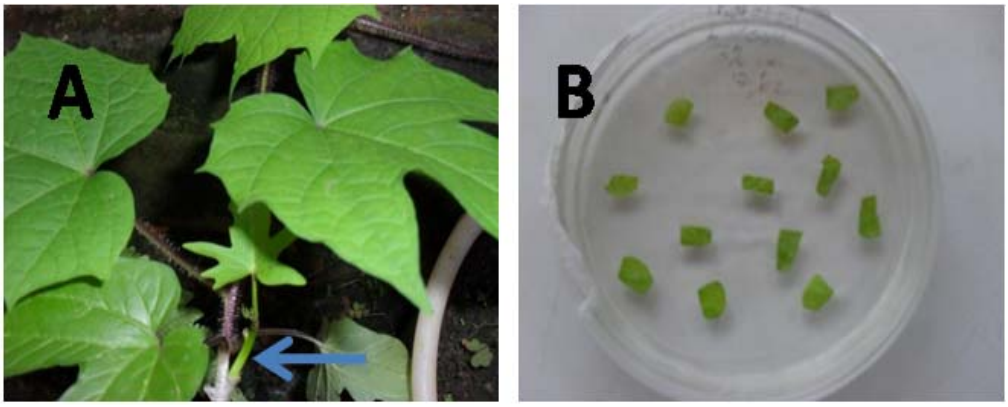

Figure 1. Disease-free explants were used for all somatic embryogenesis experiments. A: Grafted *Ipomoea setosa* with scion from cultivar Magabali. Healthy *Ipomoea setosa* leaves showing no viral infection symptoms. Arrow shows sweetpotato scion. B. Leaf explants from sterile *in vitro* cultures soon after placement on callus induction media.

Table 1. Embryogenic frequency for three Ugandan sweetpotato genotypes.

Cultivar	Organ	Explants	Callus (No)	Callus (%)	Embryogen (No.)	Embryogen. (Freq.)
Jamada	Leaf	1	1	100	1	100
Jamada	Petiole	21	17	81	13	62
Magabali	Leaf	54	50	93	39	72
Magabali	Petiole	79	58	73	21	27
Kyebandula	Leaf	19	8	42	4	21
Kyebandula	Petiole	12	3	25	1	8

Note: CIM was supplemented with 2mg 2,4-D and 5mg BAP while EIM had 3mg/L ABA.

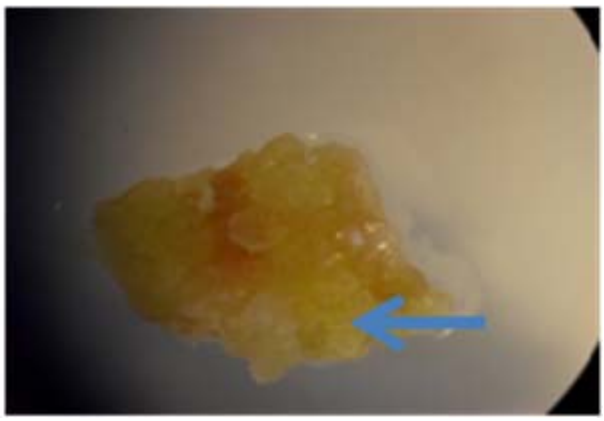

Figure 2. Enlarged section of embryogenic callus of Jamada: sections with bright yellow colour.

The embryogenic callus produced in this study has been placed on embryo induction media for maturation of the somatic embryos which could be turned into shoots on plant regeneration media. Two-way chi-square analysis showed that plant organ affects callus formation frequency. The preliminary results further show that the use of 2,4-D alone and 2,4-D with BAP had no effect. This experiment is being repeated.

Recommendation

Based on the preliminary results it is recommended that culture systems be developed which allow somatic embryogenesis to be effectively induced and maintained in a wide range of cultivars.

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