

High frequency adventitious root and shoot regeneration in recalcitrant sweet potato cultivars

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Abstract

Improvement of sweetpotato (*Ipomoea batatas*) traits like resistance to weevils through conventional hybridisation has been limited by the lack of resistance sources in the sweetpotato germplasm. The aim of this study was to investigate induction of adventitious shoots which would facilitate genetic transformation of *I. batatas* with weevil resistance genes. Media with different concentrations of thidiazuron alone or in combination with an auxin were used to investigate induction of adventitious shoots from whole leaves and stem internode segments of two *I. batatas* cultivars, Kyebandula and Bwanjule. Shoot regeneration was successfully achieved from the two explants of both cultivars. Statistical analysis showed that type of explant effect was significant ($P < 0.05$) for regenerating shoots. The results of this study are important for *I. batatas* breeding. This is particularly important for African cultivars which have hitherto been difficult to regenerate *in vitro*.

Key words: Genetic transformation, *Ipomoea batatas*, regeneration protocol, thidiazuron

Résumé

L'amélioration des spécificités de la patate douce (*Ipomoea batatas*) comme la résistance aux charançons par hybridation conventionnelle a été limitée par le manque de sources de résistance dans le matériel génétique de la patate douce. Le but de cette étude était d'étudier l'induction de pousses adventives qui faciliteraient la transformation génétique des *I. batatas* avec des gènes de résistance du charançon. Les médias avec différentes concentrations de thidiazuron seul ou en combinaison avec une auxine ont été utilisés pour étudier l'induction de pousses adventives à partir de feuilles entières et d'endiguer les segments internodaux de deux cultivars de *I. batatas*, les Kyebandula et Bwanjule. La régénération des pousses a été réalisée avec succès à partir de deux explants de ces deux cultivars. L'analyse statistique a montré que le type

d'effet d'explant a été significatif ($P < 0,05$) pour la régénération des pousses. Les résultats de cette étude sont importants pour la reproduction de *I. batatas*. Ceci est particulièrement important pour les cultivars africains qui ont été jusqu'ici difficiles à régénérer *in vitro*.

Mots clés: Transformation génétique, *Ipomoea batatas*, protocole de régénération, thidiazuron

Background

Sweetpotato (*Ipomoea batatas*) is mainly grown as a food crop in developing countries, which account for over 95% of world output. The potential production of sweetpotato in Africa as a continent is nearly 7 million tons, with all of it almost produced south of the Sahara (PRAPACE, 2003). Uganda is the biggest producer of sweetpotato in Africa and third in the world, with an annual production of 1.8 m tons (Hijmans *et al.*, 2001). Sweetpotato yield in Uganda and the rest of Africa is estimated at about 5 t/ha which is far below the potential of the region. The low sweetpotato yields in the region is to a great extent attributed to the high incidence of viral diseases and increasing attacks by weevils.

The improvement of sweetpotato traits such as resistance to weevils through conventional hybridisation has been limited by lack of sources of resistance in the available sweetpotato germplasm. Genetic transformation therefore holds potential for the improvement of this crop by offering an option of introducing important foreign genes (Kreuze *et al.*, 2008). However, successful production of transgenic sweetpotato depends on an efficient regeneration protocol (Santa-Maria *et al.*, 2009). Therefore, there is need for developing an efficient regeneration protocol for sweetpotato since present protocols are not efficient with African varieties (Luo *et al.*, 2006).

Literature Summary

Regeneration of *I. batatas* through somatic embryogenesis remains a difficult process to control. Most somatic embryogenesis protocols are cultivar dependent, difficult to reproduce, have low regeneration frequencies, and require long periods of culture and frequent media changes (Kreuze *et al.*, 2008). Thus, there is to explore other sweetpotato shoot regeneration protocols. One such protocol is based on transforming sweetpotato through regenerated adventitious shoots. This method coupled with the use of thidiazuron (TDZ) as a plant growth regulator has been used to regenerate transformed plants in many recalcitrant plant species. TDZ has

emerged as an effective bioregulant in cell and tissue cultures in a wide array of plant species (Murthy *et al.*, 1998).

Study Description

The bud induction medium was composed of Murashige and Skoog basal salts, myo-inositol (0.1 g l^{-1}), sucrose (30 g l^{-1}) and 1 ml l^{-1} sweetpotato vitamin stock. The media was adjusted to pH 5.8 before adding 3 g l^{-1} phytigel followed by autoclaving at 121°C for 15 minutes under 15 kPa. In order to assess the effect of TDZ concentration, various concentrations of filter-sterilised TDZ (0.11, 0.44 and 0.88 mg l^{-1}) were added to the medium after autoclaving. The study investigated two types of explants; intact leaves with including their petioles, and stem internode segments. These were cut from *in vitro* *I. batatas* cultures and cultured on solid bud induction medium contained in petri dishes. The culture plates were placed in the dark for 4 weeks at 25°C . After which, they were transferred and maintained under a 16 hour photoperiod under the same temperature. After every 4 weeks, the cultures were transferred to fresh medium. All experiments were laid out in a completely randomised design. Each treatment was replicated three times with each replicate having three petri dishes each containing 10 explants. Data on number of explants with adventitious shoots was collected after 12 weeks of culture. Data were subjected to analysis of variance (ANOVA) and treatment means compared using the least significant difference (LSD) test at the $P < 0.05$ level. A second experiment was carried out with the same TDZ concentrations but supplemented with $0.25 \mu\text{M}$ NAA. In this case, the effect of cultivar on shoot regeneration was not significant.

Research Application

Adventitious shoot and root regeneration of *Ipomoea batatas* was successfully achieved from recalcitrant African cultivars, Bwanjule and Kyebandula, within 12 weeks. Most explants formed callus at the cut ends within 2 weeks of placing on medium. None of the calli induced were morphogenic. This phenomenon was evident in all concentrations of TDZ. Some explants formed roots directly from the callus (Fig. 1). Regeneration of plants was possible in all the three replicates indicating that the reported protocol is reproducible. Culture medium supplemented with TDZ together with NAA increased the mean number of explants forming shoots as compared to when TDZ was used alone (Figs. 2 and 3). Stem explants performed better than leaf explants for all TDZ concentrations investigated.

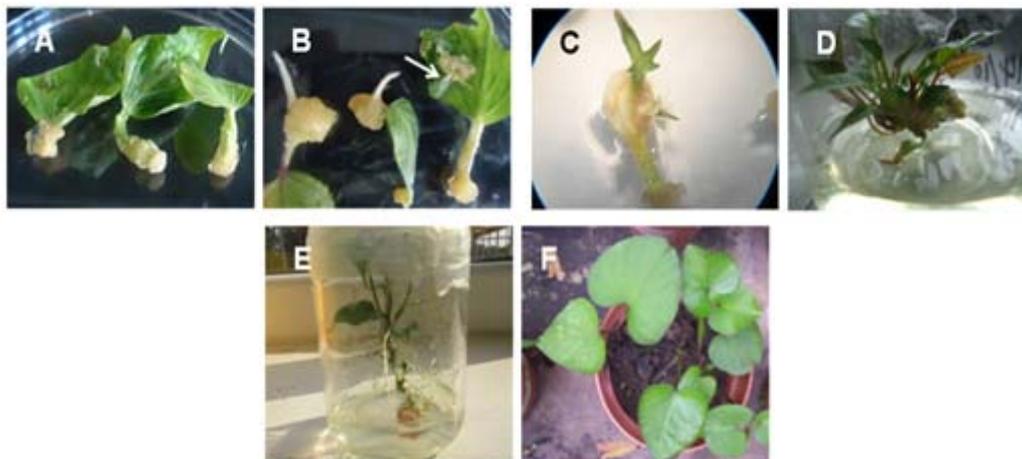


Figure 1. Adventitious shoot bud regeneration, plant development and direct root organogenesis in *Ipomoea batatas*. (a) Callus formation at the stub of petiole and adventitious bud regeneration along the sides of petiole. (b) Direct root organogenesis from callus and leaf lamina. (c) Elongation of adventitious buds leading to development of shoots. (d) Development of multiple adventitious shoots on stem internode explants. (e) Development of shoot into plantlet and emergence of roots. (f) Growth of plants in soil 4 weeks after acclimatization in the greenhouse.

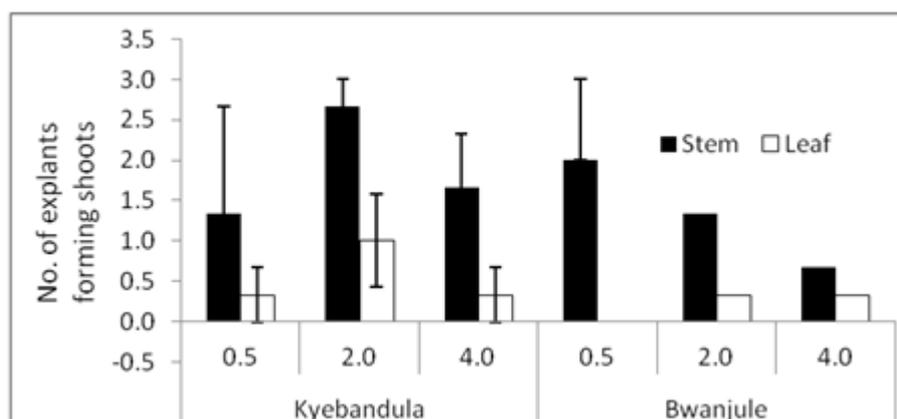


Figure 2. Effect of TDZ concentration (0.5, 2.0, 4.0 μM) on shoot regeneration frequency from stem and leaf explants of Bwanjule and Kyebandula cultivars.

Taken together, the results presented here and our preliminary experiments with somatic embryogenesis confirm that most sweetpotato cultivars that were thought to be recalcitrant can be regenerated following optimisation of media composition. This is significant because genetic transformation of potato for resistance to several stresses is potentially possible.

Recommendation

Based on the reported results it is recommended that a wider range of cultivars be tested for adventitious root regeneration.

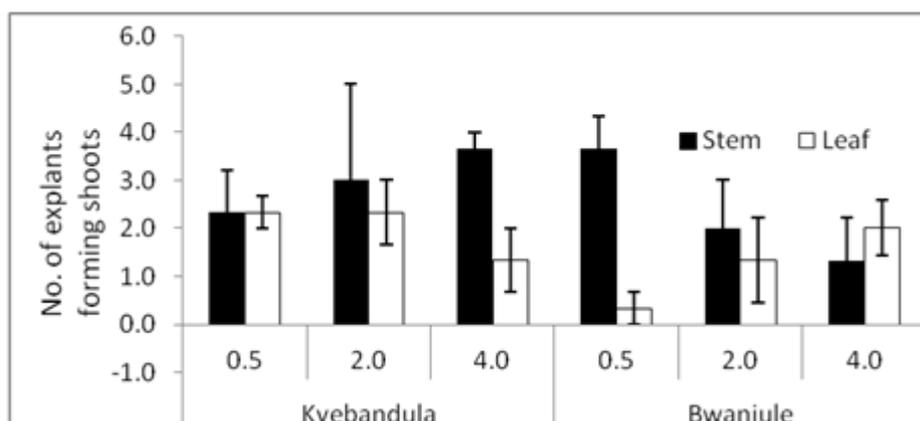


Figure 3. Effect of TDZ concentration (0.5, 2.0, 4.0 µM) in the presence of NAA (0.25 µM) on shoot regeneration frequency from stem and leaf explants of Bwanjule and Kyebandula cultivars.

Acknowledgement

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