Screening for regeneration and transformation efficiencies of African sweetpotato cultivars

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Abstract

Sweetpotato has been shown to be generally recalcitrant to genetic transformation which is a major constraint to develop biotech varieties with key traits not available in the crop's natural gene pool. Regeneration protocols have been reported for few African cultivars but their efficiency remains largely genotype-dependent. In this study, 31 African sweetpotato cultivars from CIP genebank were screened for regeneration and transformation efficiencies by organogenesis and embryogenesis, including "Jewel" and "Jonathan" varieties as organogenic and embryogenic controls respectively. First three leaves with petioles, or the three first lateral meristems were used as explants in organogenic and embryogenic experiments respectively. Regeneration by organogenesis was conducted using a two-step protocol including 2,4-D then thidiazuron, zeatin or kinetin while regeneration by embryogenesis was performed using a three-step protocol, each one using a different hormone (2,4,5-T, ABA and AG_3). More than 40% regeneration efficiencies were obtained for 6 cultivars (Imby, Kawogo, Luapula, Mafutha, Mugande and Zambezi) with organogenesis and 8 cultivars (Bwanjule, Imby, K51/3251, Luby, Malawiala, Mugande, New Kawogo and SPK004) with embryogenesis protocol after the second-step (culture media with ABA). Transformation efficiencies of these cultivars are currently estimated using GUS transient expression assay. Preliminary results show efficiencies between 30-90%. Our results suggest that our pre-screening for high regeneration and transformation efficiency has identified a dozen African cultivars amenable to genetic transformation.

Keywords: Sweetpotato, embryogenesis, organogenesis, genetic transformation.

Introduction

Sweetpotato production in Sub-Saharan Africa is mainly produced by small-scale farmers both for consumption and as a source of income (Dapaah, 1994) contributing to food security in the region. Its production in Eastern Africa yields as low as 4.17 tons as compared to its potential of 50 tons per hectare, reported in FAOstat (2006).

Constraints to production are numerous ranging from socio-economical, agronomical to biological factors. Biological constraints such as pests and diseases have been reported to cause losses between 50-100%, with weevils and virus diseases ranking the highest (Stathers *et al.*, 2005). Efforts have been made towards addressing the weevil problem with little success (Abidan *et al.*, 2005; Braun and Fliert, 1999; Dhir *et al.*, 1998; Yaku, 1992). Biotechnology offers the possibility of expanding and optimizing the use and importance of sweetpotatoes through genetic engineering, by developing a sweetpotato expressing a protein active against weevils. In order to obtain such resistant variety, a robust regeneration and transformation protocol must be established first.

Regeneration and transformation protocols have been developed for sweetpotato (Chen *et al.*, 2006; Liu *et al.*, 2001; Luo *et al.*, 2006; Sihachakr *et al.*, 1997). This crop is known to be highly recalcitrant and all protocols published so far are genotype-dependent. Genetic transformation can be achieved but for very few cultivars and with low efficiencies (Opabode, 2006). Hence, improved protocols for regeneration and transformation of African sweetpotato cultivars are in great needs.

Material and methods

Plant materials and propagation

Thirty one African sweetpotato cultivars from CIP genebank were screened for regeneration capacity using Jewel and Jonathan cultivars as controls. These cultivars are Rusenya RWA, Rusenya BDI, Mohc, Luby, Imby, Chifukama, Chihongo, Chiuva, Namagizi, Kemb10, Kemb37, Gikanda, Muibai, KSP11, SPK004, SPK013, Mafutha, Kamchiputu, Mugande, K51/3251, Malawiala, Budagala, Mwanamonde, Sinia, SPN/O, Kawogo, Bwanjule, New Kawogo, Chingowva, Luapula and Zambezi.

Organogenesis assay

The three apical leaves with petioles were used as explants, which were collected after 3 to 4 weeks from propagation. Regeneration was assayed using a two-step protocol reported by Blasco (2007).

Embryogenesis assay

Regeneration through somatic embryogenesis was assayed using the first three lateral meristems as explants. A three-step protocol having each step using a different hormone was applied (Liu *et al.*, 2001; Al-Mazrooei *et al.*, 1997; Dhir *et al.*, 1998). The initial step had MS with 2,4,5-T (in the dark), followed by MS with ABA and finally with GA_3.

Genetic transformation

Plants were infected with the hypervirulent strain EHA105 of *Agrobacterium tumefaciens* carrying the plasmid pCIP100, which differs from pCAMBIA1305.1 by conferring kanamycin resistance. This step was done according to protocols described in Medina-Bolivar *et al.* (2003), Dhir *et al.* (1998), Luo *et al.* (2006) and Xing *et al.* (2008). Genetic transformation efficiencies were assessed using a GUS expression assay following the CAMBIA protocol (www.cambia.org).

Results and discussion

Organogenesis

Regeneration commenced after three weeks for some of the cultivars while others remained recalcitrant during the two months in culture. Regeneration efficiencies expressed in percentage were calculated from the number of regenerating shoots divided by the explants. These were highly genotype-dependent ranging between 0–86% depending on the phyto-hormones used in the regeneration media. Six cultivars showed regeneration efficiencies above 40% (Table 1).

Table 1. Organogenic regeneration efficiencies for the best sweetpotato African cultivars from the CIP collection

Cultivar	CIP number	Percentage (%)
Zambezi	CIP 441772	86
Luapula	CIP 441763	60
Kawogo	CIP 440165	48
Mugande	CIP 440163	60
Mafutha	CIP 441862	46
Imby	CIP 440037	83

NB: Results are likely to change once all the cultivars have all samples included

All cultivars had swollen petioles after the auxin treatment followed by calli formation within a week; contrary to a recent protocol developed by Santa-Maria *et al.* (2009) who differed with the use of 2, 4-D on some sweetpotato varieties from the USA. Our results coincide with previous studies with African sweetpotato cultivars (Blasco, 2007; Oggema *et al.*, 2007) in that none of our hormone treatment avoided the genotype-dependence.

Embryogenesis

After two or three weeks of the explants in culture, three different type of callus were observed: pro-embryogenic callus (yellowish, compact, and slow growing), non-embriogenic callus (white or cream, friable and fast growing) and some with pro-embryogenic and non-embryogenic parts on the same callus, as reported previously by Otany & Shimada (1996).

Table 2. Efficiencies of embryogenic calli formation of the best African sweetpotato cultivars from the CIP collection

Accession name	CIP number	% embryogenic calli
K51/3251	CIP 440164	62.9
Luby	CIP 440036	46.7
Mugande	CIP 440163	43.6
New Kawogo	CIP 441745	43.5
Imby	CIP 440037	42.9
Malawiala	CIP 440172	41.5
Bwanjule	CIP 440168	40.0
SPK004	CIP 441768	40.0

NB: Results are likely to change once all the cultivars have all samples included

Eight cultivars had embryogenic calli above 40% (Table 2) in the second step of regeneration while other cultivars evaluated did not show embryogenic tissues. Inability to form embryogenic calli even using different phyto-hormones to induce embryogenesis has been reported in sweetpotatoes (Al-Mazrooei *et al.*, 1997; Triqui *et al.*, 2008).

Preliminary results of the GUS assays showed efficiencies between 30-90%. Final results of regeneration and transformation efficiencies are still pending but this step is known to be less limiting and genotype dependent than the regeneration.

In conclusion, we report here the identification of twelve sweetpotato African cultivars with workable regeneration efficiencies. This represent about one third of the genotypes tested. Preliminary results with GUS assays for these cultivars show high transformation efficiencies. We hope that using these improved protocols, researchers will be able to genetically engineer this important crop to withstand devastating production constraints, such as weevils and virus diseases.

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