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Full Length Research Paper

Induction of somatic embryogenesis in recalcitrant sweetpotato (*Ipomoea batatas* L.) cultivars

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Genetic transformation is considered as one of the most promising options for improvement of crop traits. Current transformation methods for sweetpotato depend on plant regeneration through organogenesis or somatic embryogenesis. Somatic embryogenesis and plant regeneration at a high frequency has been restricted to a few sweetpotato varieties. Three auxins namely: 2,4-dichlorophenoxyacetic acid (2,4-D), 4-fluoroamphetamine (4-FA) and 4,5-trichlorophenoxyacetic acid (2,4,5-T) were investigated in this study for enhancing somatic embryogenesis from various plant organs of recalcitrant African sweetpotato cultivars. 2,4-D was found to be the best ($p \leq 0.05$) for induction of embryogenic callus. Cultivar Bwanjule had the highest (20.2%) embryogenic callus frequency among the five African cultivars tested. The highest number of plants in this study was regenerated from the non-African cultivar variety Jonathan on media supplemented with 0.2 mg Zeatin. The emergence of roots from callus of recalcitrant Ugandan cultivars and the comparable high embryogenic responses in this work demonstrate the potential for regenerating plants from African cultivars that have not been regenerated before. The regeneration of roots in this work could be useful for the initiation of root cultures. The most important application of this work is in genetic transformation of sweet potato, particularly for improvement of resistance to weevils.

Key words: Embryogenesis, plant growth regulators, plant regeneration, *Ipomoea batatas*.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* Lam.) is a member of the family Convolvulaceae, genus *Ipomoea*, and section *batatas*. It is the only hexaploid (6x = 90) species in the *batatas* section. Sweetpotato is cultivated in tropical and sub-tropical areas of the world because of its edible tuberous roots, which are high in starch and vitamin A (Woolfe, 1991). Young leaves and vine tips are also eaten and they are an excellent source of vitamins A, C, and B_2 (Hill et al., 1992). China produces more than 80% of the world's sweetpotato followed by Nigeria and Uganda. Although sweetpotato has high economic value in developing countries, its productivity is constrained by various biotic and abiotic factors.

Weevil species, *Cylas puncticollis* Boheman and *Cylas brunneus* Fabricius, are among the most important biotic factors in Sub-Saharan Africa (Woolfe, 1991). Damage by weevils is more severe under dry conditions and production losses of 60 to 97% have been recorded

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Abbreviations: 4-FA, 4-Fluoroamphetamine; **2,4,5-T**, 4,5-trichlorophenoxyacetic acid.

during both the growth season and in storage (Stathers et al., 2003). Weevil damage results mainly from their larvae which feed within tuberous roots. Furthermore, in response to the weevil feeding, plants produce unpalatable terpenoids that render tubers unfit for consumption (Uritani et al., 1975). Breeding for weevil resistance through conventional breeding has been hampered by instability of resistance, where differences in weevil infestations have been observed among trials, locations, and seasons and at times among replicates of a single accession in a trial (Talekar, 1987). Instability of resistance has also been reported among plants in the same plot and even among storage roots within one plant (Taleker, 1987).

Genetic transformation is considered to be one of the most promising options for crop improvement because specific genetic changes can be achieved using genes from unrelated species. The first success with sweetpotato transformation was achieved by the use of *Agrobacterium rhizogenes* (Dodds et al., 1991). Since then, genetic transformation has continuously shown potential to improve sweet potato. Transgenic sweetpotato clones with improved resistance to biotic and abiotic stresses have been developed (Kreuze at al., 2008; Lim et al., 2007).

Somatic embryogenesis and plant regeneration at a high frequency has been restricted to a few genotypes. Efforts to extend this into a wide range of genotypes have shown that most of them are recalcitrant or respond at low frequencies (Al-Mazrooei et al., 1997; Anwar et al., 2010; Luo et al., 2006). Moreover regeneration protocols reported to work in some laboratories have proved difficult to reproduce in other laboratories (Moran et al., 1998). Importantly, it has been observed that African cultivars are particularly difficult to regenerate *in vitro* (Luo et al., 2006). Apart from genotype, the medium composition and type of plant organ providing the explants have been shown to influence regeneration *in vitro* (Anwar et al., 2010; Song et al., 2004). Thus, there is need to develop an efficient system of somatic embryogenesis and plant regeneration for recalcitrant African cultivars. The main aim of this study was to develop a protocol for producing high frequency of embryogenic callus which is useful in regeneration of popular African sweetpotato cultivars which, to our knowledge, have not been regenerated previously. The improvement of regeneration systems for some non-African cultivars is also reported.

MATERIALS AND METHODS

Popular Ugandan sweetpotato cultivars namely; Bwanjule, Kyebandula, Magabali, New Kawogo and Semanda were used in this study. They were provided by the Sweetpotato Program at the National Crops Resources Research Institute (NaCRRI) at Namulonge based on their wide adaptation in Uganda, high dry matter content and moderate to high resistance to sweetpotato virus disease. Three non-African varieties namely; Huachano from Peru, and Jonathan and Jewel from the U.S.A were included. The three non-African varieties were previously reported to regenerate through embryogenesis or organogenesis (Cipriani et al., 1999; Kreuze et al., 2008; Luo et al., 2006). The selected cultivars were grown in a screen house at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) and used to supply vines for initiation of *in vitro* cultures.

In vitro culture initiation

Vigorously growing vines containing five to eight nodes without lateral branches were collected from screen house grown plants. All leaves were cut from the vines followed by washing thoroughly in running tap water and Teepol™ detergent to remove dirt. The vines were then taken to laminar flow chamber. The vines were surface sterilized with 39 % commercial JIK containing 3.85% NaOCl) and 0.01% of Tween 20® for 20 min. This was followed by submerging vines in 70% ethanol for 2 min for further disinfection and also to remove the corrosive sodium hypochlorite. After surface sterilization, the vines were transferred to sterile water and rinsed three times followed by cutting into one or two nodes and insertion into sweetpotato propagation media.

Sweetpotato propagation medium was made by mixing 4.3 g/L premix of Murashige and Skoog, 1962 (MS) salts 30 g/L sucrose, 5 ml/L sweetpotato vitamin stock comprised of 40 g/L ascorbic acid, 20 g/L L-arginine, 4 g/L putrescine HCl, 2 mg/L gibberellic acid (GA_3) and 0.4 g/L calcium pantothenate. The media was adjusted to pH 5.8 before adding 3 g/L phytagel followed by autoclaving at 121ºC for 15 min under 15 kPa. *In vitro* plants cultured on this media were used to supply whole leaf, leaf discs and petiole explants for somatic embryogenesis experiments as described below.

Callus induction

Three to four weeks old petioles, leaf discs and whole leaves (petiole with lamina) from *in vitro*-grown plants were tested on callus induction media. The Ugandan cultivars used in this study are Bwanjule, Kyebandula, Magabali, Semanda and New Kawogo. The callus induction media was made of 4.3 g/L MS premix (without vitamins), 1 ml/L Vitamins stock (0.5 mg/ml nicotinic acid, 0.1 mg/ml thiamine and 0.5 mg/ml pyridoxine), 100 mg/L myo-inositol, 30 g/L sucrose and 7 g/L agar. The media was divided into three equal parts and a different type of auxin was added to each part. The three different auxins tested in this study were 1 mg/L 4-fluorophenoxyacetic acid (4FA), 1.3 mg/L 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). These concentrations were derived from previous studies on sweetpotato somatic embryogenesis (Anwar et al., 2010; Cipriani et al., 2001; Song et al., 2004; Triqui et al., 2007). All auxins were filter-sterilized and added to autoclaved media that was cooled to about 60°C. The cultures were transferred to fresh media every two weeks. Data on number of explants forming callus and those forming embryogenic callus was collected after 12 weeks. Embryogenic callus was identified as being compact and light green or bright yellow while non-embryogenic calli was white and friable and formed earlier and faster than embryogenic callus (Ciprianin et al., 1999).

Plant regeneration

After observing that 2,4-D induced high frequency embryogenic callus than 4-fluoroamphetamine (4-FA) and 2,4,5-T, the callus

Table 1. Plant regeneration media and amount growth regulators added to each.

Type of regeneration media*	Plant growth regulator added to media (mg/L)			
	Zeatin riboside	2,4-D	GA_3	Abscisic acid
F9	0.2	-	-	-
G24D	-	0.05	0.1	-
F25	-	-	-	1
PGR-free	-	-	-	-

*All media listed contain 4.3 g/L MS salts, 0.1 g/L myoinositol, 30 g/L sucrose, 1 ml vitamin stock and pH adjusted to 5.8 before adding 3.0 g/L phytagel. Filter-sterilized PGRs were added to all media after autoclaving and cooling to 60°C.

induction protocol of Cipriani et al. (2001) was adopted. The embryogenic callus from three most promising Ugandan cultivars namely New Kawogo, Bwanjule and Magabali and non-African cultivars Jewel, Jonathan and Huachano were used to investigate effect of various medium in inducing plant regeneration from embryogenic callus. Young whole leaves were placed on semisolid medium (0.43 g/L MS salts, 20 g/L D-glucose, 7 g/L agar, 0.5 g/L 2-N-morpholino-ethanesulfonic acid (MES), 0.1 ml vitamins stock, 0.1 mg/L naphthalene acetic acid (NAA), 1 mg/L benzylaminopurine (BAP), 2 mg/L gibberellic acid (GA_3), adjusted to pH 5.5) and incubated at 28ºC for 2 days. The explants were then transferred to F15 medium (4.3 g/L MS salts, 0.05 mg/L 2,4-D, 0.2 mg/L zeatin riboside, 0.1 g/L myo-inositol, 30 g/L sucrose, 1 ml/L vitamin stock and 3.0 g/L phytagel) for three days, followed by transfer to F9 medium (Table 1). The explants were transferred to fresh F9 media once in every two weeks for callus induction (Kreuze et al., 2008). Instead of transferring all the resulting embryogenic callus from F9 to G24D medium as reported by Cirpiani et al. (2001), the callus were separated and placed on four types of media; F25, F9, plant growth regulator free (PGR-free) and G24D medium (Table 1). These adjustments were made at this stage following preliminary observations of root emergence before shoot regeneration on G24D medium. Emerging shoots on the various media were taken to fresh F9 medium followed by multiplication on sweetpotato propagation medium and transferred to a screenhouse at the National Agricultural Research Laboratories (NARL) at Kawanda, Uganda.

Statistical analysis

All cultures were incubated in the growth chamber at 28°C with a photoperiod of 16 h. A total of 10 explants were used per treatment in each Petri dish, and this was replicated three times and arranged in a completely randomized design with genotype, type of plant organ and type of plant growth regulator (PGR) as factors. Statistical analysis for the effect of the three factors on total callus proliferation and formation of embryogenic callus was performed using general linear model of analysis of variance (ANOVA) at 5% level of significance. Mean values were compared using the least significance difference (LSD) method at the 5% level of significance. Two-way Chi-square was used to establish the contribution of PGR to plant regeneration from embryogenic callus.

RESULTS

Conditions affecting proliferation of embryogenic callus

All cultivars, plant organs and auxins investigated in this study induced both embryogenic and non-embryogenic callus (Figure 1). Callus proliferation was noticed within five days in all experiments. The frequency of total callus (embryogenic and non-embryogenic callus) was not significantly different ($p \leq 0.05$) among all cultivars. In general, cultivar Bwanjule had the highest frequency (51.3%) of callus while Semanda had the lowest (40.3%) frequency (Figure 2). However, both the Plant growth regulator (PGR) and plant organ were found to have a significant effect on total callus induced when investigated within cultivars. PGR had a very high significant ($p \leq 0.05$, Table 3; Figure 4) effect on callus induction frequency.

All the three factors namely auxin type, plant organ and cultivar had a significant ($p \leq 0.05$) effect on the frequency of embryogenic callus. The auxin found to be best for induction of both total callus and embryogenic callus for all the 5 Ugandan genotypes investigated in this study was 2,4-D (Figure 3). Mean comparisons (LSD, $p < 0.05$) for total callus induction showed that petioles and whole leaves were not significantly different although the callus produced from petioles of all cultivars was higher than for both whole leaf and leaf disc (Figure 5). Similar results were found when means of different plant organs were investigated for their contribution to proliferation of embryogenic callus.

Plant regeneration from embryogenic callus

Chi-square analysis showed that PGR had a significant ($p \leq 0.05$) effect on plant regeneration. Cultivar Jonathan regenerated on all the four types of media F25, F9, G24D and PGR-free (Table 2). The highest number of plants was regenerated in F9 media (Table 2). A similar trend was observed for cultivar Huachano. Except for F25, all the other media led to the production of roots directly from callus in cultivar Huachano and Jonathan, with G24D media leading to high production of roots (Figure 6). The production of roots was observed in all cultivars, except for Bwanjule, and high frequency of roots was produced on G24D media for all cultivars, except for cultivar Jewel (Table 2).

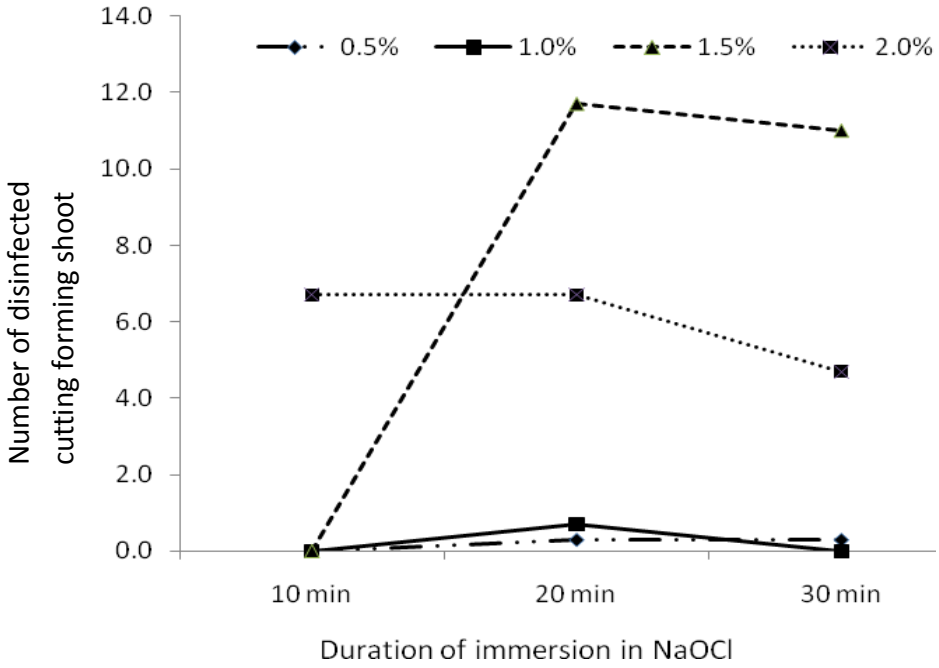

Figure 1. Effect of duration of immersion (minutes) and concentration (v/v) of sodium hypochlorite (NaOCl) on number of disinfected vines able to form shoots in propagation media after 3 weeks. Mean ± standard error, with 15 cuttings per experiment replicated 3 times.

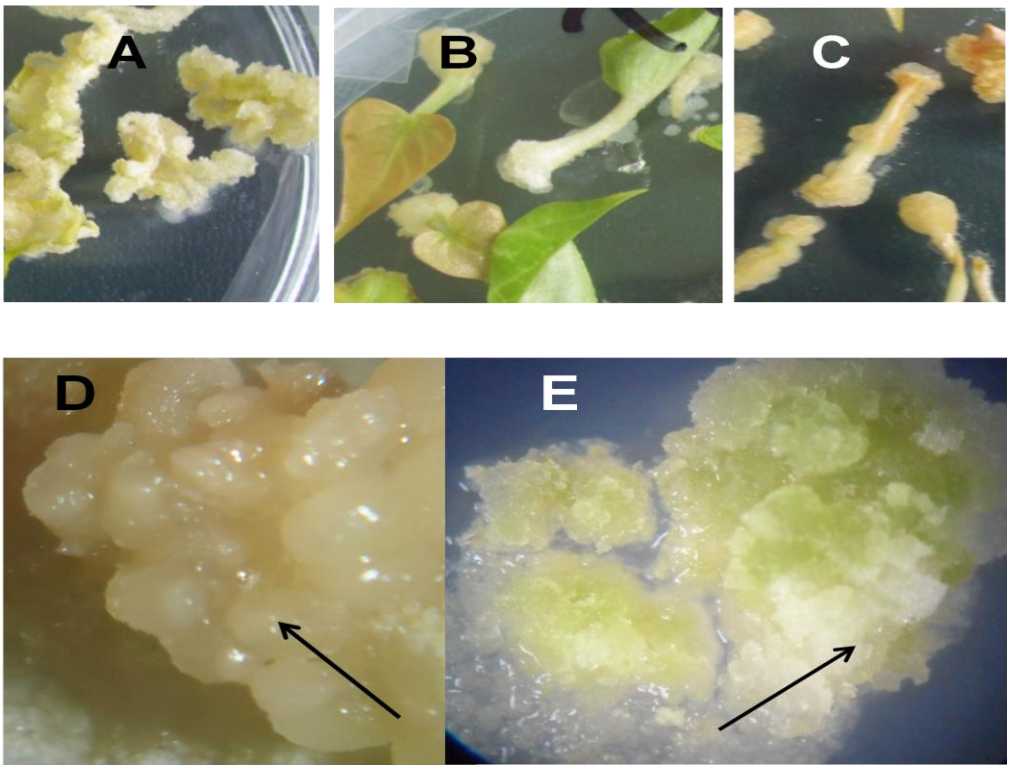

Figure 2. Callus formation from different types of explants. (A) Leaf disc explants. (B) Whole leaf explants. (C) Petiole explants. (D) Compact bright yellow embryogenic sections of callus (arrow). (E) White and friable non-embryogenic sections of callus (arrow).

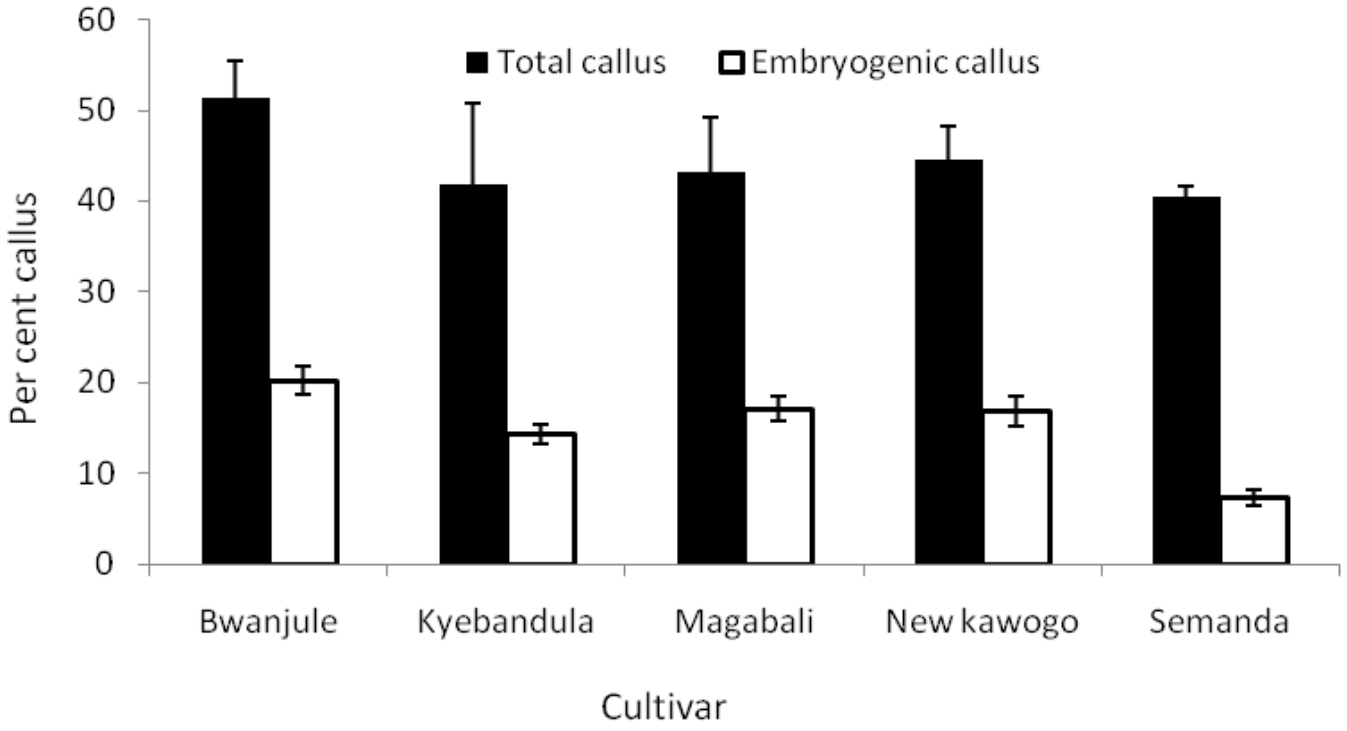

Figure 3. Effect of plant genotype providing explants for callus induction and formation of embryogenic callus (expressed as a percent of total explants tested). Means ± standard error, with 10 explants per Petri dish replicated 3 times.

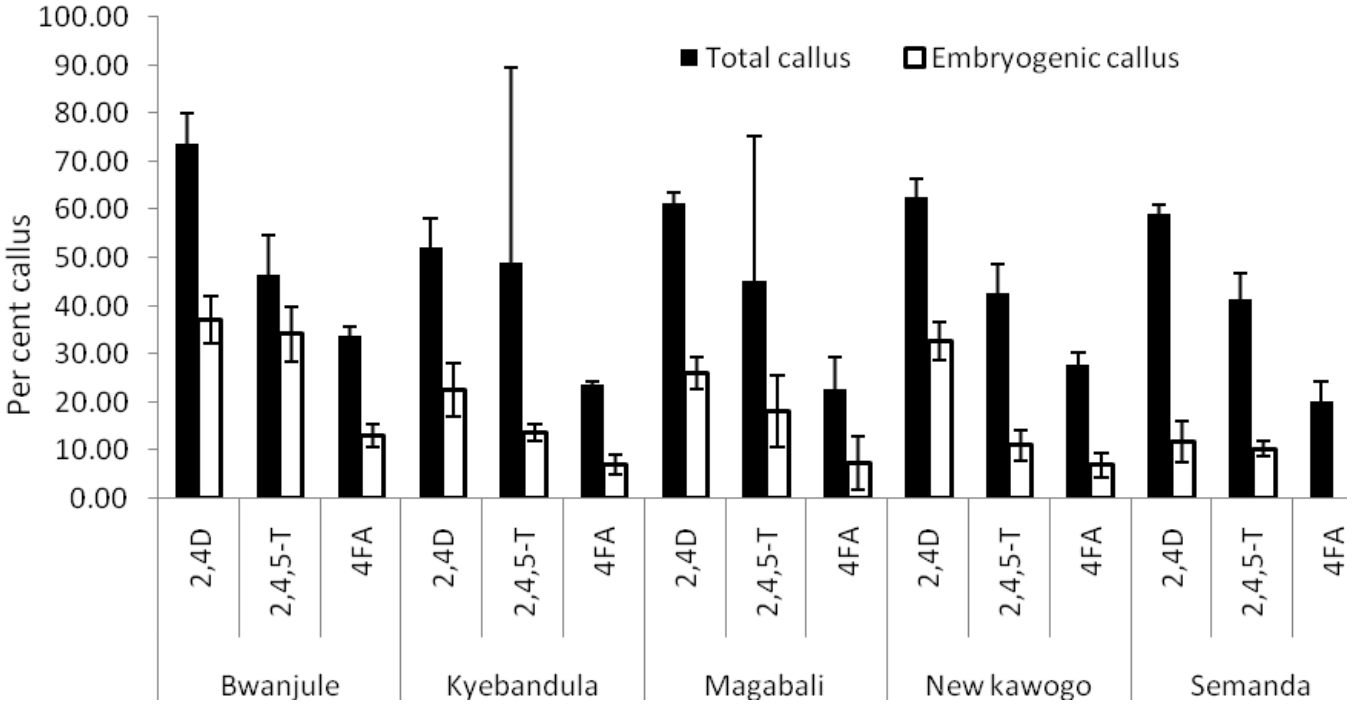

Figure 4. Effect of auxin on induction of callus and formation of embryogenic callus on explants (expressed as a percent of total explants tested). Per cent were calculated callus formation embryogenic callus formation. Means ± standard error, with 10 explants per Petri dish replicated 3 times within cultivar.

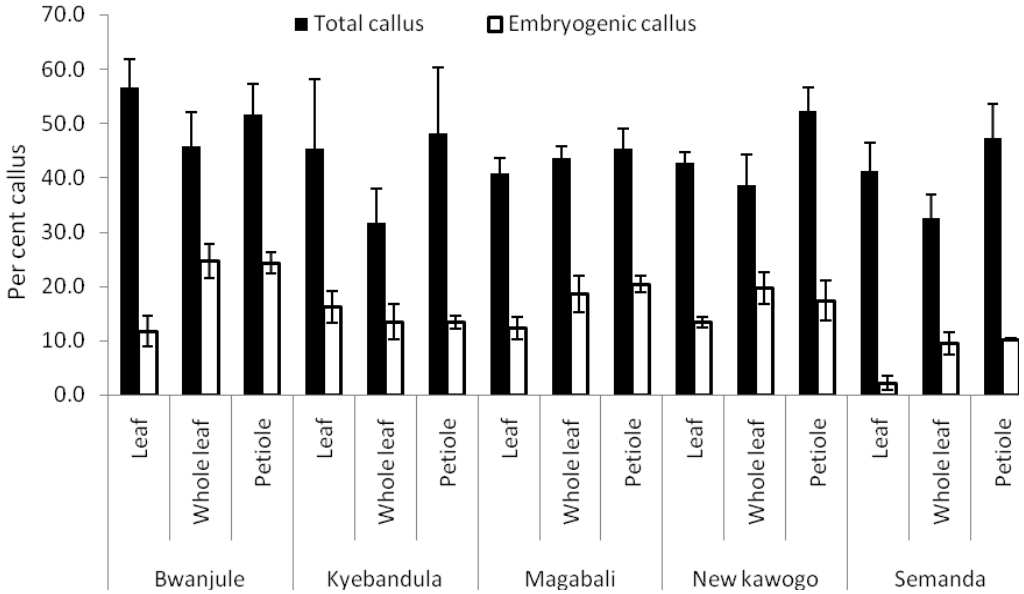

Figure 5. Effect of type of plant organ supplying explants for callus induction and formation of embryogenic callus (expressed as a percent of total explants tested). Means ± standard error, with 10 explants per Petri dish replicated 3 times within cultivar.

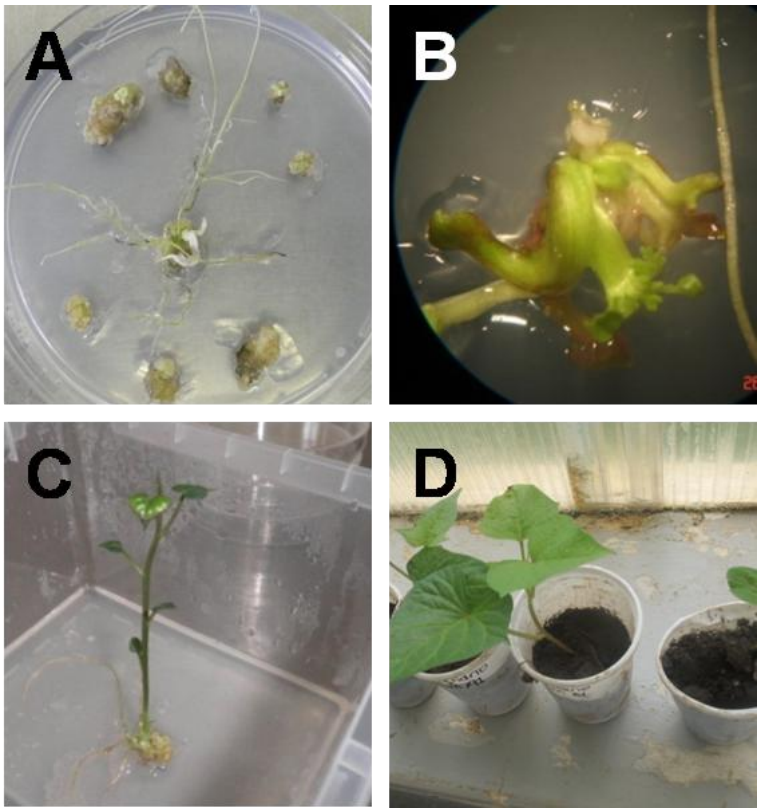

Figure 6. Regeneration of sweetpotato from embryogenic callus. (A) Formation of roots directly from callus. (B) Emergence of amorphous shoots from embryogenic tissue removed from callus and placed directly on regeneration media. (C) Growth of shoots and formation of leaves on shoots emerging from embryogenic callus. (D) Growth of regenerated plants after transfer from media to soil.

Table 2. Effect of type of medium on sweetpotato regeneration from embryogenic callus originating from whole leaf.

Sweetpotato variety*	Type of medium	Number of callus clusters that produced shoots	Number of callus clusters that produced roots
Huachano	F25	0	0
	F9	4	2
	PGR-free	1	2
	G24D	1	4
Jonathan	F25	3	0
	F9	7	5
	PGR-free	2	2
	G24D	1	6
Jewel	F25	2	0
	F9	3	0
	PGR-free	1	2
	G24D	1	0
New Kawogo	F25	0	0
	F9	0	2
	PGR-free	0	0
	G24D	0	3
Bwanjule	F25	0	0
	F9	0	0
	PGR-free	0	0
	G24D	0	0
Magabali	F25	0	0
	F9	0	3
	PGR-free	0	1
	G24D	0	6

*A total of 10 embryogenic callus masses were tested for each type of medium and sweetpotato variety.

DISCUSSION

The present study was initiated with the aim of developing a protocol for induction of embryogenic callus from recalcitrant African cultivars. These cultivars have not been reported in previous regeneration and transformation work. In general, and particularly in important African sweetpotato cultivars, an efficient regeneration system has been very difficult to establish (Luo et al., 2006). Other reports show that there is a correlation between the quality and quantity of embryogenic callus and subsequent plant regeneration in sweetpotato (Al-Mazrooei et al., 1997). Therefore it is important to first establish a system for somatic embryogenesis for the recalcitrant cultivars to facilitate regeneration and enable application of genetic engineering to improve important traits (Anwar et al., 2010; Moran et al., 1998).

In order to establish a procedure for induction of high frequency of somatic embryogenesis that could be used in genetic transformation experiments, various plant organs and plant growth regulators that were previously reported in successful sweetpotato transformation were tested. Up to five popular Ugandan cultivars were investigated since previous reports consistently show that cultivar has a high influence on somatic embryogenesis (Al-Mazrooei et al., 1997). Somatic embryogenesis was selected as a method of regeneration in this study because it results in high efficiency of selection during transformation since each whole plant originates from a single transformed cell (Sihachakr et al., 1997). It is important that plant regeneration is of single cell origin, avoiding possibility of chimeras or escapes after genetic transformation (Gong et al., 2005).

In this work, the auxin 2,4-D was identified as the best auxin for inducing embryogenic callus. There were

Table 3. ANOVA showing mean sum of squares and F- probability for frequency of callus and embryogenic callus generated by different varieties, organ type and plant growth regulators.

Source of variation	df	Callus (frequency)		Embryogenic callus (frequency)	
		MS	F- probability	MS	F- probability
Cultivar	4	0.12497	0.274	1.5055	0.002
PGR	2	3.11263	< 0.001	6.7132	< 0.001
Plant organ	2	0.3251	0.038	0.1482	0.628
Cultivar X PGR	8	0.10124	0.4	0.1714	0.823
Cultivar X Plant organ	8	0.14285	0.171	0.2796	0.535
PGR X Plant organ	4	0.19015	0.103	2.1552	< 0.001
Cultivar X PGR X Plant organ	16	0.17737	0.036	0.4934	0.097
Residual	90	0.09576		0.3168	

MS, Murashige and Skoog.

significant ($p \leq 0.05$) differences in the effect of the auxins for production of embryogenic callus (Table 3). Various reports demonstrate the best embryogenic response from medium containing 2,4-D (Cipriani et al., 2001; Sihachakr et al., 1997). However other workers report that 2,4,5-T is better than 2,4-D in inducing embryogenic callus (Al-Mazrooei et al., 1997; Triqui et al., 2007). Successful regeneration of transgenic plants from five Japanese cultivars has also been reported when 4-FA was used while there was no success with 2,4-D (Anwar et al., 2010). The auxin concentrations investigated in this study were determined from those previous studies that showed successful embryogenesis over a wide number of sweetpotato cultivars (Anwar et al., 2010; Cipriani et al., 2001; Song et al., 2004; Triqui et al., 2007).

Although type of plant organ explanted did not influence the frequency of total callus, as much as plant growth regulator did, it was found to have a highly significant effect on the quality of the induced callus. In general 'petiole with lamina' explants were able to induce the highest frequency of embyogenic callus, although in some cultivars the results were comparable with 'petiole' explants. Importantly, all the shoots and roots regenerated in this work only emerged from callus of 'leaf with petiole' explants. In recent studies of induction and regeneration of adventitious shoots from sweetpotato, it was found that petiole explants were the least responsive as compared to both leaf and stem explants (Gong et al., 2005).

Plants were regenerated from cultivar Huachano within five months in the present study. This contrasts with the reported regeneration system by Kreuze et al. (2008) took 12 months to regenerate this cultivar. The reduction of time to regenerate plants is of high significance since long time culture of callus-derived plants increases the chance for somaclonal variation. Other researchers have reported rapid regeneration of Jewel through organogenesis (González et al., 2008; Luo et al., 2006). Although these workers regenerated plants within 10 weeks, they encountered the presence of escapes when polymerase chain reaction (PCR) was used to examine transformed plants regenerated through organogenesis. In contrast, workers who used somatic embryogenesis have reported 100% transformation frequency among all regenerated plants although the number of plants regenerated was low (Song et al., 2004).

Although the number of plants produced in the present study is low, the protocol demonstrates reproducibility for the reported cultivars since plants were regenerated using embryogenic callus obtained from three different experiments. A reproducible protocol is more important than regenerating a high number of plants in a single experiment (Yu et al., 2007). Low numbers of regenerated plants have been reported in many previous studies with sweetpotato cultivars from different geographical areas (Otani et al., 2003).

The repetitive regeneration of cultivars Huachano, Jonathan and Jewel in a short time through somatic embryogenesis in this study is a critical breakthrough although some researchers regenerated these popular cultivars previously. It is difficult to reproduce regeneration results from one experiment to the next or even from one laboratory to another. Moran and co-workers (1998) reported that many protocols that had previously been reported to lead to plant regeneration did not produce good results in their hands. The inconsistencies in regeneration responses within the same cultivar may be due to a variation in the developmental and physiological stage of *in vitro* plants, affecting the cultural behaviour of explants (Jones et al., 2007; Triqui et al., 2007). For instance, the sweetpotato cultivar Duclos 11 which had shown ability to regenerate plants from protoplast-derived callus (Sihachakr and Ducreux, 1987) did not give any embryogenic response when lateral buds were used later (Sihachakr et al., 1997).

The production of roots from callus of cultivars Huachano, Jonathan and Jewel in media supplemented with 2,4-D may be useful for the initiation of root cultures,

which may serve as a source of tissue for regeneration of plants or hairy root cultures (Jones et al., 2007). The regeneration of shoots was not possible following the protocol of Cipriani et al. (2001) who placed embryogenic callus on G24D medium. Plants were only regenerated in this work when minor modifications were made to the protocol of Cipriani et al. (2001) as follows; placement on F25 (ABA), F9, G24D (2,4-D and GA_3) and PGR-free medium (Table 1). Both ABA and GA_3 are required after somatic embryos have been formed but have been found to have a negative effect on regeneration if applied earlier (George et al., 2008). In some reports ABA has been used for synchronization and maturation of pre-formed somatic embryos of sweetpotato while GA_3 has been shown to elongate pre-formed structures (Anwar et al., 2010; George et al., 2008; Song and Yamaguchi, 2006)

In this study, the highest number of plants was regenerated on F9 media (Table 2). The production of roots was observed in all cultivars, except cultivar Bwanjule, and high frequency of roots was observed in G24D media except for cultivar Jewel (Table 3). Except for Bwanjule and New Kawogo, all cultivars produced roots directly from callus on medium without PGR. The reason for regeneration of roots on PGR-free medium is not straightforward although it is possible that there were high levels of endogenous auxins in the explanted organs (Becerra et al., 2004; George et al., 2008).

In conclusion, this study has identified growth regulators, plant organ type and some popular African cultivars for high production of embryogenic callus, a major constraint to regeneration through somatic embryogenesis. Additionally, by successfully reducing the time for regeneration of the non-African cultivar Huachano, the present study has overcome a major setback in regeneration through somatic embryogenesis. Despite the lack of development of shoots from the tested African cultivars, the emergence of *de novo* roots from callus and comparably high embryogenic responses show the potential of regenerating plants from recalcitrant African cultivars. Importantly, the production of roots in this work may be useful for the initiation of root cultures, which may serve as a source of tissue for regeneration of plants or hairy root cultures. The culture conditions optimized in this study are currently being used in *Agrobacterium*-mediated genetic transformation of Ugandan sweetpotato with genes that have potential to regenerate transgenic sweetpotato resistant to weevils.

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REFERENCES

Al-Mazrooei S, Bhatti MH, Henshaw GG, Taylor NJ, Blakesley D (1997). Optimisation of somatic embryogenesis in fourteen cultivars of sweetpotato [*Ipomoea batatas* (L.) Lam.]. Plant Cell Rep. 16:710-714.

Anwar N, Watanabe KN, Watanabe JA (2010). Transgenic sweetpotato expressing mammalian cytochrome P_{450}. Plant Cell Tiss. Org. Cult. 105:219-231.

Becerra DC, Forero AP, G´ongora GA (2004). Age and physiological condition of donor plants affect *in vitro* morphogenesis in leaf explants of *Passiflora edulis f. flavicarpa*. Plant Cell Tiss. Org. Cult. 79:87-90.

Cipriani G, Fuentes S, Bello V, Salazar LF, Ghislain M, Zhang DP (2001). Transgene expression of rice cysteine proteinase inhibitors for the development of resistance against sweetpotato feathery mottle virus. International Potato Center, Lima, pp. 267-271.

Cipriani G, Michaud D, Brunelle F, Golmirzaie A, Zhang DP (1999). Expression of soybean proteinase inhibitor in sweet potato. International Potato Center, Lima, pp. 271-277.

Dodds JH, Merzdorf C, Zambrano V, Sigüeñas C, Jaynes J (1991). Potential use of agrobacterium-mediated gene transfer to confer insect resistance in sweet potato. In: Sweetpotato Pest Management: a Global Perspective. Oxford: West View Press.

George EF, Hall MA, de Klerk G-J (2008). Plant propagation by tissue culture: Springer, Dordrecht. The Netherlands.

Gong Y, Gao F, Tang K (2005). *In vitro* high frequency direct root and shoot regeneration in sweetpotato using the ethylene inhibitor silver nitrate. South Afr. J. Bot. 71:110-113.

González RG, Sánchez DS, Guerra ZZ, Campos JM, Quesada AL, Valdivia RM, Arencibia AD, Bravo KQ, Caligari PDS (2008). Efficient regeneration and *Agrobacterium tumefaciens* mediated transformation of recalcitrant sweetpotato (*Ipomoea batatas* L.) cultivars. Asia. Pac. J. Mol. Biol. Biotechnol. 16.

Hill WA, Bonsi CK, Loretam PA (1992). Sweetpotato research: Current status and future needs. In: Sweetpotato technology for the 21st century. Tuskegee, Alabama Tuskegee University.

Jones MPA, Yi Z, Murch SJ, Saxena PK (2007). Thidiazuron-induced regeneration of Echinacea purpurea L.: Micropropagation in solid and liquid culture systems. Plant Cell Rep. 16:13-19.

Kreuze JF, Klein IS, Lazaro MU, Chuquiyuri WC, Morgan GL, Mejía PGC, Ghislain M, Valkonen JPT (2008). RNA silencing-mediated resistance to a crinivirus (Closteroviridae) in cultivated sweetpotato (*Ipomoea batatas* L.) and development of sweetpotato virus disease following co-infection with a potyvirus. Mol. Plant Pathol. 9:589-598.

Lim S, Kim YH, Kim SH, Kwon SY, Lee HS, Kim JS, Cho KY, Paek KY, Kwak SS (2007). Enhanced tolerance of transgenic sweetpotato plants that express both CuZnSOD and APX in chloroplasts to methyl viologen-mediated oxidative stress and chilling. Mol. Breed. 19:227-239.

Luo HR, Santa Maria M, Benavides J, Zhang DP, Zhang YZ, Ghislain M (2006). Rapid genetic transformation of sweetpotato (*Ipomoea batatas* (L.) Lam) via organogenesis. Afr. J. Biotechnol. 5:1851-1857.

Moran R, Garcia R, Lopez A, Zaldua Z, Mena J, Garcıa M, Armas R, Somonte D, Rodriguez J, Gomez M, Pimentel E (1998). Transgenic sweetpotato plants carrying the delta-endotoxin gene from *Bacillus thuringiensis* var. tenebrionis. Plant Sci 139:175-184.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant 15:473-497.

Otani M, Wakita Y, Shimada T (2003). Production of herbicide-resistant sweetpotato (*Ipomoea batatas* (L) Lam.) plants by *Agrobacterium tumefaciens*-mediated transformation. Breed Sci. 53:145-148.

Santa-Maria M, Pecota KV, Yencho CG, Allen G, Sosinski B (2009). Rapid shoot regeneration in industrial 'high starch' sweetpotato (*Ipomoea batatas* L.) genotypes. Plant Cell Tiss. Org. Cult.109:109-117.

Sihachakr D, Ducreux GC (1987). Plant regeneration from protoplast

culture of sweetpotato (*Ipomoea batatas* Lam.). Plant Cell Rep. 6:326-328.
Sihachakr D, Haicour R, Cavalcante Alves JM, Umboh I, Nzohge D, Servaes A, Ducreaux G (1997). Plant Regeneration in sweetpotato (*Ipomoea batatas* L., Convolvulaceae). Euphytica 96:143-152.
Song G, Yamaguchi K (2006). *Sweetpotato [Ipomoea batatas (L.) Lam.]. In*: Agrobacterium Protocols. Second Edition. Volume 2. Edited by Kan Wang. :Humana Press Inc., 999 Riverview Drive, Suite 208, Totowa, New Jersey 07512.
Song GQ, Honda H, Yamaguchi KI (2004). Efficient *Agrobacterium tumefaciens*–mediated transformation of sweetpotato (*Ipomoea batatas* (L.) Lam.) from stem explants using a two step kanamycin-hygromycin selection method. 40:359-365. *In vitro* Cell Dev - Plant 40:359-365.
Stathers TE, Rees D, Nyango A, Kiozya H, Mbilinyi L, Jeremiah S, Kabi S, Smit N (2003). Sweetpotato infestation by Cylas spp. in East Africa: II. Investigating the role of root characteristics. Int. J. Pest Man. 49:141-146.
Talekar NS (1987). Feasibility of the use of resistant cultivars in sweetpotato weevil control. Insect Sci. Applic. 8:815-817.
Triqui ZEA, Guédira A, Chlyah A, Chlyah H, Souvannavong V, Haïcour R, Sihachakr D (2007). Effect of genotype, gelling agent, and auxin on the induction of somatic embryogenesis in sweetpotato (*Ipomoea batatas* Lam.). Plant Biol. Pathol. 331:198-205.
Uritani I, Saito T, Honda H, Kim WK (1975). Induction of furanoterpenoids in sweetpotato roots by the larval components of the sweetpotato weevils. Agric. Biol. Chem. 37:1857-1862.
Woolfe JA (1991). Sweet potato: An Untapped Food Resource:Cambridge University Press, Cambridge. England.
Yu B, Zhai H, Wang Y, Zang N, He S, Liu Q (2007). Efficient *Agrobacterium tumefaciens*-mediated transformation using embryogenic suspension cultures in sweet potato, *Ipomoea batatas* (L.) Lam. Plant Cell Tiss. Org. Cult. 90:265-273.