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The effect of 2,4-D on callus induction using leaf lobe of sweet potato as a source of explant

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Key words: Callusogenesis, 2, 4-dichlorophenoxyacetic acid, CLC/ *Ipomoea* medium, leaf lobes.

Abstract

The effect of 2, 4-dichlorophenoxyacetic acid (2,4-D) on young leaf lobes of three sweet potato accessions UE007, UK-BNARI and SA-BNARI for callus induction was investigated. Callusogenesis was achieved when leaf lobe explants from four weeks old healthy growing plantlets of three sweet potato accessions SA-BNARI, UK-BNARI and UE007 were cultured on CLC/ *Ipomoea* medium supplemented with 1.0 - 4.0 mg/l 2,4-D with 4.0 mg/l 2,4-D being the optimal concentration. However, the calli were non-embryogenic and therefore could not produce embryos when transferred to 0.1 mg/l BAP amended medium but rather produced either single or multiple shoots. The highest percentage shoot (83.3 %) was obtained from 4.0 mg/l 2,4-D-derived callus. These results indicate that indirect shoot development via a callus phase is a feasible option for sweet potato propagation using *in vitro* techniques.

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Introduction

Conventional propagation of sweet potato involves the use of vine cuttings or root or adventitious root "slips" that grow from the tuberous roots during storage period to propagate the crop. Both cuttings and the tubers are obtained from previous cropping season and their selection is based on quality of tubers and physical appearance of the propagules. Conventional propagation of sweet potato most often results in slow growth and poor survival rate (Akoroda, 2009). The vegetative mode of propagation also leads to the transmission of viral and other systemic diseases as they are transmitted from generation to generation leading to drastic reduction in tuber yield (Badoni and Chauhan, 2010). Since the viral diseases are transmitted via cuttings, there is the need to develop alternate *in vitro* regeneration methods to produce disease-free planting materials for both subsistence and large scale sweet potato farmers.

In vitro propagation of sweet potato can be achieved via direct organogenesis (meristem culture or nodal culture) or indirect organogenesis (callus culture) or (somatic embryogenesis) (Barka, 2009; Oggema *et al.*, 2007). Both methods (direct and indirect organogenesis) ensure rapid clonal multiplication and year round production of disease-free propagules of elite clones.

Callus is very useful in *in vitro* cultures as it provides a fundamental step in regenerating many recalcitrant crops. It can be multiplied in culture for indefinite period even in absence of light. Callus also can be used for long term conservation of plant tissues by maintaining them under slow growth conditions, and as target tissue for genetic transformation (Ali *et al.*, 2007). Callus culture is often initiated by culturing explants on a medium supplemented with a combination of appropriate growth regulators (El Far *et al.*, 2009). Therefore, this paper sought to report on the effect of 2, 4 D on callus induction using young leaf lobes of sweet potato.

Materials and methods

Experimental Site and planting material

The experiment was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra-Ghana. Four weeks old *in vitro* plantlets of sweet potato accession UE007, SA-BNARI and UK-BNARI were used for this study. Plantlets of these accessions were maintained on solid Murashighe and Skoog (1962) basal medium supplemented with 0.25 mg/l BAP and 0.1 mg/l α-naphthaline acetic acid (NAA), and 0.1 mg/l gibberellic acid (GA_3).

Culture media

The culture media used was sweet potato CLC/ Ipomoea medium (Chee *et al.*, 1992) for callus induction. The medium was supplemented with 30 g/l sucrose, 1 mg/l myoinositol, 1 mg/l $CuSO_4$, and 3.5 g/l and 0 (control), 1.0, 2.0, 3.0 or 4.0 mg/l 2, 4-D according to the experimental requirement. The pH of the media was adjusted to 5.8 using either 1 M NaOH or 1 M HCl. The media were gelled with 3.5 g/l phytagel prior to autoclaving at a temperature of 121^{o}C and 15 psi pressure for 15 minutes. . Fifteen (15 ml) milliliter of the medium was dispensed into Petri dishes or honey jars (50 ml) depending on the experiment and stored at room temperature or in a refrigerator.

Culture conditions

Cultures were incubated in dark by placing them in light proof box in the growth room. Cultures were examined for callus formation 21 days after culture and the number of explants that developed callus was recorded. All induced calli were transferred to honey jar containing 50 ml of CLC/ *Ipomoea* medium supplemented with 0.1mg/l BAP and transferred to growth room lighting conditions at a temperature of 27 ± 2 ^{o}C, with a photoperiod of 16 h light and 8 h darkness, and a light intensity of 2700 lux provided by white fluorescent tube.

Plantlet regeneration from callus culture

Leaf lobe explants from four weeks old healthy growing plantlets of three sweet potato accessions SA-BNARI, UK-BNARI and UE007 were dissected under the microscope and cultured on sweet potato 15ml of CLC/ Ipomoea medium (Chee *et al.*, 1992) for callus induction in Petri dishes. Each Petri dish contained three leaf lobe explants which constituted a treatment which was replicated four times. The pH of the medium and autoclaving were as described. Cultures were incubated in conditions as described above. Cultures were examined for callus formation 21 days after culture and the number of explants that developed callus was recorded. All induced calli were transferred to honey jar containing 50 ml of CLC *Ipomoea* medium supplemented with 0.1 mg/l BAP and transferred to growth room lighting conditions as mentioned above. The number of calli that developed shoots, mean number of shoots per plant were counted after three weeks of culture in the light.

Results

Plant regeneration via callus induction

Leaf lobe explants of all the sweet potato accessions cultured on CLC/ Ipomoea medium supplemented with different concentration of 2, 4-D developed calli within 9 to 15 days after culture except on a hormone free medium where there was no callus development even after 21 days of culture in the dark. The size of calli produced from all the three accessions varied according to the concentration of 2,4-D in the culture medium (Table1). The size of callus developed by explants cultured on CLC/*Ipomoea* medium supplemented with 1.0 mg/l 2,4-D was small in size compared with those cultured on 4.0 mg/l 2,4-D which was approximately three times that of those induced on 1.0 mg/l 2,4-D (Figure 1). Also, the colour of the calli varied according to the concentration of 2,4-D in the culture medium. While those induced on 1 mg/l were brownish, those produced on 4 mg/l D were greenish in colour (Figure 1C) suggesting the formation of shoot primordia.

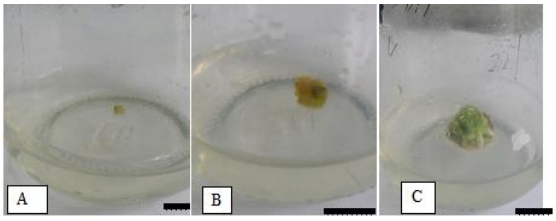

Fig. 1. Calli formation on leaf lobe explants of SA-BNARI cultured on (A) 1.0 mg/l 2,4-D, (B) 3.0 mg/l 2,4-D and C 4.0 mg/l 2,4-D after 21 days of culture in dark (Bar A= 1 mm, Bar B=8 mm Bar C=12 mm).

The percentage calli formation from the leaf lobe explants also varied according to the concentration of the growth regulator in the culture medium ranging from 50 to 100 %. However, statistical analysis did not show any significant differences between the interaction of the accession and hormone. Generally, the percentage calli formation on medium supplemented with 1.0 or 2.0 mg/l 2,4-D was low ranging from 50 to 75 % while those cultured on 3.0 or 4.0 mg/l 2,4-D was high ranging from 75 to 100%. All leaf lobe explants (100%) cultured on CLC/ *Ipomoea* medium supplemented with 4.0 mg/l 2,4-D developed calli independent of the accessions.

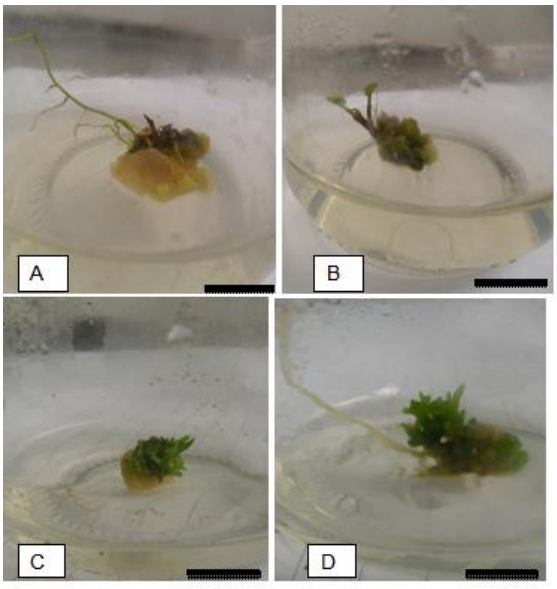

Fig. 2. Shoot development after three weeks of culture in light (A): SA-BNARI and (B): UK-BNARI formed on 1mg/l 2,4-D showing single shoot development (C): UE007 and (D): UK-BNARI shoots formed on 4mg/l 2,4-D showing multiple primordial shoots) (Bars = 10 mm).

Shoot development from calli

The transfer of calli to 0.1 mg/l BAP amended medium resulted in single or multiple rosette-like shoots (Figure 2). The percentage of calli with shoots increased as the concentration of the 2,4-D in the callus initiation medium increased (Table 2). The concentration of 2,4-D in the callus initiation medium significantly affected shoot development. In almost all the accessions, the percentage calli shoots on a medium with 4mg/l 2,4-D significantly differed from the remaining treatments.

Similarly, the mean number of shoots per callus also significantly ($P<0.05$) increased from the lowest concentration of 2,4-D to the highest. Of the three accessions studied, the local accession UE007 developed more shoots per callus varying from 2.5 to 8.7 while SA-BNARI developed the least (2.5 to 6.3).

Table 1. Effect of different concentrations of 2,4-D on calli size and percentage calli induction in leaf lobes of three sweet potato accessions after 21 days of culture in the dark.

2,4-D mg/l	*Degree of callus formation*	*Percentage calli induction (%)*		
		SA-BNARI	UK-BNARI	UE007
Control	-	0^{a}	0^{a}	0^{a}
1	+	50^{b}	75^{bc}	75^{bc}
2	+	75^{bc}	75^{bc}	50^{b}
3	++	75^{bc}	100^{c}	100^{c}
4	+++	100^{c}	100^{c}	100^{c}

Values in the same column followed by same superscripts are not significantly different at ($P<0.05$) according to Tukey's pair wise comparison. Number of leaf lobes cultured per treatment is 12

Note:

- indicates no callus formed,

+ low callus formation,

++ moderate callus formation,

+++ indicates high callus formation.

Table 2. Effect of different concentrations of 2,4-D on shoot development and mean number of shoots per callus in three sweet potato accessions after three weeks of culture in light.

	SA-BNARI			*UK-BNARI*			*UE007*		
2,4-D mg/l	Number of calli transferred	% calli with shoots	Mean number of shoots per callus	Number of calli transferred	% calli with shoots	Mean number of shoots per callus	Number of calli transferred	% calli with shoots	Mean number of shoots per callus
0	0	0.0^{a}	0.0^{a}	0	0.0^{a}	0.0^{a}	0	0.0^{a}	0.0^{a}
1	6	50.0 ± 0.2^{b}	2.5 ± 1.4^{ab}	9	41.7 ± 0.2^{b}	1.9 ± 1.4^{ab}	9	50.0 ± 0.2^{b}	2.5 ± 1.4^{ab}
2	9	58.3 ± 0.2^{b}	3.8 ± 1.4^{bc}	9	58.3 ± 0.2^{b}	3.3 ± 1.4^{b}	6	50.0 ± 0.2^{b}	2.7 ± 1.4^{ab}
3	12	58.3 ± 0.2^{b}	2.4 ± 1.4^{ab}	12	75.0 ± 0.2^{bc}	5.8 ± 1.4^{bcd}	12	75.0 ± 0.2^{bc}	6.1 ± 1.4^{bcde}
4	12	75.0 ± 0.2^{bc}	6.3 ± 1.4^{bcde}	12	83.3 ± 0.2^{c}	6.8 ± 1.4^{de}	12	75.0 ± 0.2^{bc}	8.7 ± 1.4^{e}

Values in the same column followed by same superscripts are not significantly different at($P<0.05$) according to Tukey's pair wise comparison.

Discussion

Plant regeneration via callus induction

Callus induction in plant species is not only used for transformation of crop species via *Agrobacterium* (Otani *et al.*, 2003; Newell *et al.*, 1995) transformation or particle bombardment (Chugh and Khurana, 2002) but also for plant regeneration. Thus, in this study, callusogenesis was used as an alternative mode of regeneration in three sweet potato accessions. Synthetic growth regulator (2,4-D) has been used extensively to induce both embryogenic and non embryogenic callus in many plant species such as cassava (Danso *et al.*, 1999) and para rubber (KumariJayasree *et al.*, 1999) due to its ability to cause dedifferentiation in plant tissues. The dedifferentiation process is characterised by changes in the metabolic activities, disappearance of storage products and rapid cell division resulting in the development of callus prior to expression of embryogenic competence or organogenesis (Feher *et al.*, 2003). The addition of 2,4-D caused callus formation from leaf lobe, however, the intensity of the callus increased as the concentration of the growth regulator increased in the CLC/ *Ipomoea* medium. Kobayashi and Shikata (1966) has reported that the ability of 2,4-D to induce calli depends on the crop as well as the concentration of the auxin. In this study, the optimal concentration to achieve callus induction was 3 or 4 mg/l. Liu *et al.* (1997) reported that, MS supplemented with 2 mg/l 2, 4-D was optimal for callus formation in sweet potato varieties. In this report, 4 mg/l 2,4-D was optimal for callus induction. The differences between the present report and that of Liu *et al.* (1997) is that while in the present study CLC medium was used, Liu *et al.* (1997) used MS medium. Soomro and Memon (2007) used as low as 0.2 mg/l 2,4-D to achieve 100 % calli formation in hypocotyl explants of *Jatrophacurcas*(*L.*). All these reports suggest that the genotype, the type of explants and the concentration of growth regulator 2,4-D play critical role in callus formation.

The calli produced were non embryogenic as they failed to produce somatic embryo when they were transferred to 0.1 mg/l BAP embryo maturation medium. However, the calli developed greenish structures indicating shoot primordial formation which led to the development of shoots. These shoots were either single or multiple shoots depending on the concentration of the auxin in the callus induction medium. Such indirect shoot organogenesis has been observed in several species including sugarcane (*Saccaharum officinarum*) (Ali *et al.*, 2007), sweet potato (*Ipomoea batatas*) (El Far *et al.*, 2009; Tsay and Tsang, 1979) and innala (*Solenostemon rotundifolius*) (Prematilake, 2005) which have been incorporated into the micropropagation production systems.

Conclusion

2,4-Dichlorophenoxy acetic acid (2,4-D) at a concentration of 4 mg/l induced callus in all the sweet potato accessions. However, the calli induced were not embryogenically competent and therefore could not produce somatic embryo but rather produced shoots. Hence, this procedure could be used to complement *in vitro* methods of conservation of sweet potato as well as the production of propagules for both subsistent and commercial farmers.

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