



EFFECT OF GENOTYPE AND 2,4-D CONCENTRATION ON CALLOGENESIS IN SUGARCANE (*SACCHARUM SPP.* HYBRIDS)

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ABSTRACT

The effect of genotype and 2, 4-D on callogenesis was investigated in sugarcane (*Saccharum spp.*) hybrid cultivars; SP726180, B47419, M1176/77 and M2119/88. To evaluate the response of the genotypes to callus induction and embryogenic callus production, leaf base explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 2.5, 3.0, 3.5, or 4.0mg/L 2,4-D for 8wks. While, the number of responding explants was found to generally increase with the increase in 2,4-D concentration from 2.5 to 4.0mg/L, embryogenic callus production was highest on media supplemented with 3.0 and 2.5mg/L. There were no significant differences in the number of responding explants and embryogenic callus production among B47419, M1176/77 and M2119/88, these genotypes however exhibited a significantly higher response when compared to SP726180 indicating their high propensity to *in vitro* callogenesis.

Key words: Sugarcane genotypes, Leaf explant, 2, 4- Dichlorophonoxyacetic acid (2, 4-D), Callogenesis

INTRODUCTION

Sugarcane (*Saccharum sp.*) is an important crop for many tropical and subtropical countries which account for approximately 75% of global sugar production (Chengalrayan and Gallo-meagher, 2001). In Nigeria, total consumption of sugar was put in 2000 as 700,000 tonnes per annum and growing at a rate of 7% (David, 2000). Presently, the nation's sugar needs are met by almost 100% imports. According to Bugaje and Mohammed (2007), the already established sugarcane estates are operating at only 17% capacity, largely due to low per hectare yields of the existing genotypes.

Reasons for this low yield are poor seed multiplication procedure, which takes 6-7years to multiply an improved variety to commercial scale and the crop's susceptibility to attacks by pathogens which cause significant yield reduction. As sugarcane is propagated vegetatively, infections are easily transferred from one generation to another and farmers usually end up with infected planting material. For this reason, sugarcane seed multiplication using tissue culture is an efficient and suitable means of producing disease-free planting material. Application of biotechnology techniques could also have a significant impact on the genetic improvement of traits in sugarcane.

An important pre-requisite in achieving these objectives, is the ability to establish a morphogenic culture under *in vitro* condition. In sugarcane, this can be achieved through both somatic embryogenesis and organogenesis. Since embryogenic culture allows for cyclic recovery of more plants, regeneration via somatic embryogenesis is an attractive alternative for rapid clonal propagation of sugarcane. The ability to regenerate plantlets from callus tissue of *Saccharum*

species was first demonstrated by Heinz & Mee (1969). Since then, callus culture in sugarcane has been widely reported (Lakshmann, 2006). The general strategy is to induce callus formation from explants on Murashige and Skoog (Murashige and Skoog, 1962) medium supplemented with 2,4-D or Picloram, and subject the callus cells to embryogenesis by withdrawing or reducing the concentration of the auxin. However, a major limitation of this callus system is the development of embryogenic callus portions among highly proliferating non-embryogenic callus tissue. This process requires repeated subculture for the selection of embryogenic callus which increases the chances of somaclonal variation. As these limitations have become unavoidable, strategies to improve production of embryogenic callus from cultured explants is necessary. The objective of this study was to assess the callogenesis in four important commercial sugarcane cultivars in response to 2,4-D treatment.

MATERIALS AND METHODS

Four (4) sugarcane (*Saccharum spp.*Hybrids) genotypes; SP726180, B47/419, M2119/88 and M1176/77 were used in this study. Growing tips of 6 month old plants grown in the screening plots were used as source of explants. Leaf cylinders provided by immature leaf rolls and apical meristem were used for callus induction. Apical portions of healthy shoots were stripped to the terminal bud and washed with sterilized distilled water and surface sterilized by initial dipping in 70% ethanol (v/v) for 2 minutes followed by treatment with 20% (commercial bleach containing 5% Sodium hypochlorite) for 20 minutes, thereafter the materials were rinsed 3 times in sterile double distilled water.

The outer immature leaf rolls were removed under aseptic condition using a sterile forcep and surgical knife. The explants were cut into 10mm segments and planted on 11cm diameter petri dishes containing 10ml of sterilized culture medium consisting of MS basal medium supplemented with 3% sucrose, 100mg/l myo-inositol and 2.5, 3.0, 3.5 or 4.0mg/l⁻¹ 2, 4-D, then solidified with 8% agar before autoclaving at 121°C for 15 minutes. Ten (10) explants were cultivated in each petri dish and five petri dishes were used per treatment. Cultures were incubated in the dark for 4 weeks, after which each petri dish was examined to determine the number of explants forming callus. The resulting calli were transferred to fresh medium for further callus proliferation. After eight (8) weeks of culture in the presence of 2, 4-D, distinctions between embryogenic and non-embryogenic callus was examined on the basis of callus external aspect (Nabors *et al.*, 1983). Embryogenic callus was of glossed aspect, compact and characterized by its white to cream colour and its nodular structure, while non-embryogenic callus was of wet aspect, translucent and brownish in colour. The number of explants forming callus and frequency of embryogenic callus were recorded and the data was expressed as percentage callus formation and percentage embryogenic callus production respectively.

RESULTS AND DISCUSSION

The explants cultured without 2,4-D turned brown and died within a few days of culture, while incubation of explants on media containing auxin resulted in callus formation in all the sugarcane varieties. Swelling and subsequent unwhorling of the explants was observed after 2-3 days of culturing on MS medium supplemented with 2,4-D. The explants turned pale in colour and a creamy white callus initiated after 2 weeks from the cut edges and injured sites and gradually spread all over the explants. In sugarcane culture, 2,4-D has proved to be indispensable for callus induction, proliferation and even embryogenesis (Brisible *et al.*, 1994, Chengalrayen and Gallo-meagbar, 2001, Kenia, *et al.*, 2006). A range of 2,4-D concentrations (2.5-4.0mg/L) were evaluated for callus induction and embryogenic callus production (Table 1). In all the genotypes highest percentage of explants forming callus was recorded with 3.5mg/L of 2,4-D and callus formation slightly decreased when 2,4-D was increase to 4.0mg/l (83.7%) and progressively decreases with decrease in the 2,4-D concentration. The promotive effect of 2.5 to 4.0mg/L of 2, 4-D for callus induction and proliferation in sugarcane was frequently reported (McCallum *et al.*, 1998; Somashekhar *et al.*, 2000; Javed

et al., 2001, Ali *et al.*, 2008). Although callus induction was higher when MS was supplemented with 3.5 and 4.0 mg/L, embryogenic callus production was significantly higher when MS was supplemented with 3.0mg/L (83.75%) and 2.5mg/L (81.25%) (Fig.1). The same concentrations were reported optimum for embryogenic callus production in sugarcane by many authors (Khan *et al.*, 1998 and 2004). The high specificity for 2,4-D for callogenesis in sugarcane could be attributed to the presence of putative 2,4-D receptors (auxin-binding protein) present on the surface of cell membrane of the explant. It is beleived that 2,4-D plays an important pivotal role in the dedifferentiation of somatic cells into embryogenic callus cells. Michalczuk *et al.*, (1992) reported that culture of explant in 2, 4-D containing medium, increases the endogenous auxin levels in the explants. Polar transport of the endogenous auxins (IAA) is essential for the establishment of bilateral symmetry during embryogenesis in monocot (Fisher and Neuhaus, 1996). The efficacy of 2.5 and 3.0mg/L 2,4-D in the induction of embryogenic callus in sugarcane , proved that, these concentrations are optimum for stimulating considerable increase in the IAA levels in the cultured explant.

The response of the sugarcane genotypes to callus induction and embryogenic callus production was also evaluated (Fig.2). Optimum response to callus induction was recorded in M1176/77 (65.00%), B47419 (61.00%) and M2119/88 (60.75%) and were significantly higher than SP726180 (45.75%). Genotypic response to embryogenic callus production demonstrated that, M2119/88, B47419 and M1176/77 exhibited optimum embryogenic callus production of 50.50%, 49.00% and 51.50% respectively, while SP726180 exhibiting a significantly lower embryogenic callus production of 32.75%. This variation in the response of sugarcane genotypes to *in vitro* callogenesis could be attributed to the physiological differences, particularly the endogenous hormones levels. Endogenous hormones levels were postulated to be the main difference between genotypes with various grades of embryogenic competence in sugarcane (Bhaskaran and Smith, 1990). In conclusion, 2.5 and 3.0mg/L 2, 4-D were found to be optimum for embryogenic callus induction and this effect could be attributed to their activity on the endogenous hormone levels. It is suggested that, this activity might be the secret behind achieving the induction of high frequency embryogenic callus and subsequent plant regeneration in many sugarace genotypes.

Table 1: Responce of sugarcane genotypes to callus formation under different concentrations of 2, 4-D.

2,4-D conc. (mg/L)	M2119/88 %CF	B47419 %CF	SP726180 %CF	M1176/77 %CF	Morphotype
0.00	0.00	0.00	0.00	0.00	-
2.50	42.50	45.00	31.25	50.00	cr, w, b
3.00	83.75	87.50	62.50	85.00	cr, w, b
3.50	90.00	87.50	67.50	95.00	cr, w, b
4.00	87.50	85.00	67.50	95.00	cr, w, b
-	%EC	%EC	%EC	%EC	-
0.00	0.00	0.00	0.00	0.00	w cr, c
2.50	95.00	87.50	52.50	90.00	w,cr, c
3.00	87.50	90.50	65.00	92.50	w, cr ,c
3.50	42.50	40.00	27.50	42.50	w, cr, c
4.00	27.50	27.00	18.75	32.50	w, cr,c

Key: CF=Callus,Formation, EC=Embryogenic Callus, cr=Cream, w=white, b=brown, c=compact, Morphotype=callus Morphological type

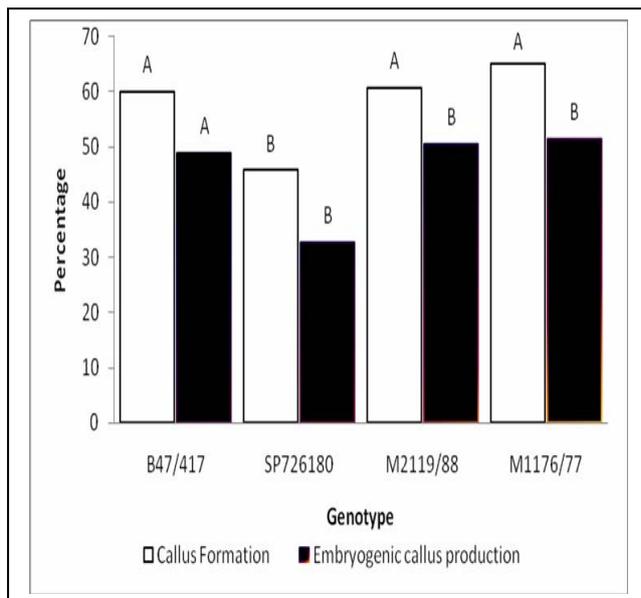


Figure 1: Effect of 2,4-D on callus formation and embryogenic callus production.

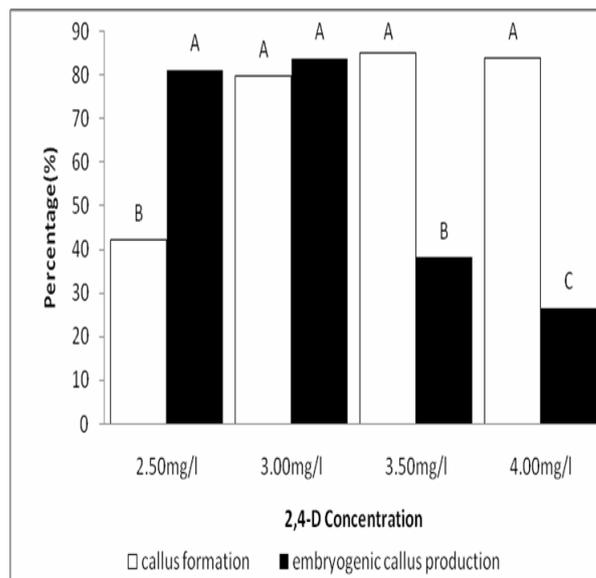


Figure 2: Response of sugarcane morphotypes to callus formation and embryonic callus production

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