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Differential in *vitro* Response of Sugarcane (*Saccharum officinarum* L.) Genotypes for Callogenesis

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ABSTRACT: Six genotypes of sugarcane namely BO147, BO146, BO110, CoP9301, BO130 and BO91 were analyzed for their *in vitro* responses. Scaly leaf culture of all the cultivars on the medium MS basal + 2, 4-D (3.0 mgl^{-1}) + 5% sucrose resulted in callogenesis including embryogenic callus. The highest frequency of callus formation was found in the cultivar, BO147 (94.4%) whereas lowest in the cultivar, CoP9301 (58.33%). The excellent growth of callus was observed in the cultivar, BO147, while poorest in the cultivar, CoP9301. Different colour of callus, greenish cream, brownish cream, whitish cream, brownish and creamish yellow was observed in the different cultivars. The highest frequency of cultures showing embryogenic callus formation was found in cultivar, BO147 (70.58) whereas the lowest in CoP9301 (28.57). Cytological study of the embryogenic callus showed metabolically active meristemoid regions which may lead to organogenesis. A genotypic difference for in vitro responses was found in the six selected cultivars with BO147 showing the best response and CoP9301 the lowest. Thus, the sugarcane cultivars showed differential response under *in vitro* conditions suggesting development of specific callus induction protocol for individual genotype.
Keywords: Sugarcane, Scaly leaves, MS media, Callogenesis, Embryogenic callus.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L; 2n= 80 to 205) is the chief source of sweetening agent worldwide. Commercially it is cultivated from stem cutting with each cutting or set having two or three buds. The lack of multiplication procedures is the major bottleneck in varietal development programme. Since it requires enough seed of elite breeding line during initial stages of replicated field trial and also after release of variety, it takes about 8-10 years to spread a new variety. To solve the slow multiplication rate, micropropagation holds great potential for mass multiplication and subsequent rejuvenation and quality production (Heinz and Mee, 1969). Moreover the introduction of a given genotype under *in vitro* selection programme depends on its aptitude to *in vitro* culture, particularly to callus induction and embryogenic callus production (Badawy *et al*; 2008) . In fact, for several species, studies have shown that genotype affects plant *in vitro* culture responses (Abe and Futsuhara, 1984; Mikami and Kinoshita, 1988; Van Sint Jan *et al*; 1990; Arzans and Mirodjagh, 1999, Burner (1992). Keeping in view the importance of genotype dependent in vitro responses the present study was formulated with the objective to analyze, the *in vitro* response of the six improved varieties of sugarcane towards callogenesis and embryogenic callus formation.

MATERIALS AND METHODS

Field grown six improved sugarcane varieties *viz.* BO147, BO146, BO110, BO91, CoP9301 and BO130 were collected for explant source. The disease free healthy 4-5 month old sugarcane tops were selected. Young leaves were removed from the top portion of plant and the spindle was excised from the top. The collected scaly leaves explants were partially trimmed off and then washed thoroughly under running tap water for 10 minutes each of three cycles to wash off external dust/contaminant. Thereafter, spindle was again washed with a liquid detergent 1 ml savlon solution with 50 ml distilled water for 2 minutes. After this treatment, explants were washed again under running tap water for 10 minutes.

Pre- treatment: - washed explants were pre-treated in a mixture of solution of 0.5% ascorbic acid, 0.5% citric acid, 0.1% streptomycin and 0.1% bavistin for 15 –20 minute to avoid the oxidation of leached phenolic compound and microbial contaminants.

Surface sterilization: - pre-treated explants were surface sterilized with 0.2% mercuric chloride solution and potassium chloride subsequently for 3-4 minutes and washed with sterile distilled water 3-4 times inside laminar flow.

Inoculation of explant: - prepared explants were then inoculated inside laminar flow under sterilized condition and transferred to incubation room at 25^0 + -2^0c with relative humidity 50-80% and light 2 kilo lux

Preparation of media: - Murashige and Skoog (1962) medium was used as basal medium supplemented with 2, 4-D (3.0 mgl^{-1}) and 5% sucrose for various responses.

Callogenesis: - The frequencies of callogenesis in the cultured scaly leaves were calculated out of total established culture tubes. Embryogenic callogenesis was confirmed by the morphological features such as compact nature, green colour and presence of globular structures. To confirm the embryogenic nature of callus, it was regularly fixed into 3:1 ethanol and acetic acid fixative stained with acetocarmine and observed cytologically. Photographs of cultures were taken on Nikon-Coolpix L18, 8.0 mega pixels, Model No. L18 (HK) BK and Olympus-Digital SLR camera. Model No. E- 330 (Japan).

Statistical analysis: Standard deviation (SD) and standard error (SE) were calculated using Microsoft-7 excel formulas

RESULTS AND DISCUSSION

The response of cultured explant of sugarcane was observed as establishment, callogenesis and embryogenic callus formation. Cultured scaly leaves of the six selected cultivars showed callogenesis on reported media MS basal + 2, 4-D (3.0 mgl^{-1}) + 5% sucrose. The callus was initiated after 11 to 23 days of inoculation. The callogenesis was earliest in cultivar, BO147 (11-15 days), followed by BO146 (13-15days), BO130 (15-17days), BO91 (16-18 days) and BO110 (19-21), while delayed in the cultivar, CoP9301 (20-23days). The frequency of callus formation was highest in the cultivar, BO147 (94.4%), followed by BO146 (88.23%), BO130 (82.35%), BO91 (81.25%) and BO110 (73.33%), while the lowest in the cultivar, CoP9301 (58.33%) (Fig.1and Fig. 2).

Callus parameters *viz*. nature, growth and colour of all the genotypes are shown in (Table. 1 & Fig. 2). The growth of callus was poor to excellent. The excellent growth of callus was observed in the cultivar, BO147, good in cultivar BO146, BO110 and BO130, moderate in BO91, whereas poorest in the cultivar, CoP9301.The colour of callus observed was greenish cream in the cultivar, BO147 brownish cream in BO146, whitish cream in BO110, brownish in CoP9301, and creamish yellow in both the cultivars, BO130 and BO91. The nature of callus was found friable with some compact region in the cultivar BO147, BO110 and BO91, compact in the cultivar BO146 and CoP9301, whereas the friable callus was observed in the cultivar, BO130. The highest frequency of cultures showing

embryogenic callus formation was found in cultivar, BO147 (70.58), followed by BO130 (64.28), BO110 (63.63), BO146 (60.0) and BO91 (53.84), whereas the lowest in CoP9301 (28.57). These results were in accordance with the results of Burner (1992) who reported the genotype effect on callus production and plant regeneration by analyzing the *in vitro* response of three sugarcane genotype to callus production from mature caryopsis and by measuring their regeneration rate. Gandonou *et al* (2005b) studied similar effect of genotype on callus production and plant regeneration from leaf explants of sugarcane. They used nine sugarcane genotypes for evalution of their callus induction capacity, embryogenic callus production and plant regeneration ability. Badawy *et al* (2008) reported the response of three genotype of sugarcane for callus induction and embryogenic callus production and their *in vitro* salt tolerance.

Beside these reports the genotype has been considered as an important factor determining the type and magnitude of responses in tissue culture (Kumari, 2000).The genotype may show their differences for tissue culture responses through dominant and additive effect of nuclear and cytoplasm factors (Peng and Hodges, 1989).Thus, a genotypic difference has been found in tissue culture responses of many plant (Kumar and Mazumdar, 1988; Kumar and Verma, 1988; Choudahry and Kumar, 1999). Genotype of sugarcane also played an important role in determining type and magnitude of tissue culture responses. The difference in tissue culture responses in genotype BO110, BO120, BO91 and BO128 of sugarcane have also been reported by Grasim and Boung (1989) and Jimenez *et al* (1990).

Callus cytology

The cytological study of the developed embryogenic calli was done in all the six selected genotypes of sugarcane (Fig.3). The callus cells were generally enlarged and had some cytoplasm and nucleus. These cells were characterized by the presence of large vacuoles. Among these callus cells, some started dividing and organized themselves into a group of metabolically active smaller cells having dense cytoplasm and prominent nucleus. These cells constituted meristemoid which could be transformed into different types of organogenesis including shoots.

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).

Fig.1. Comparative response of callus induction in selected cultivars of sugarcane on MS basal + 2, 4-D (3.0 mgl^{-1}) + 5% sucrose medium

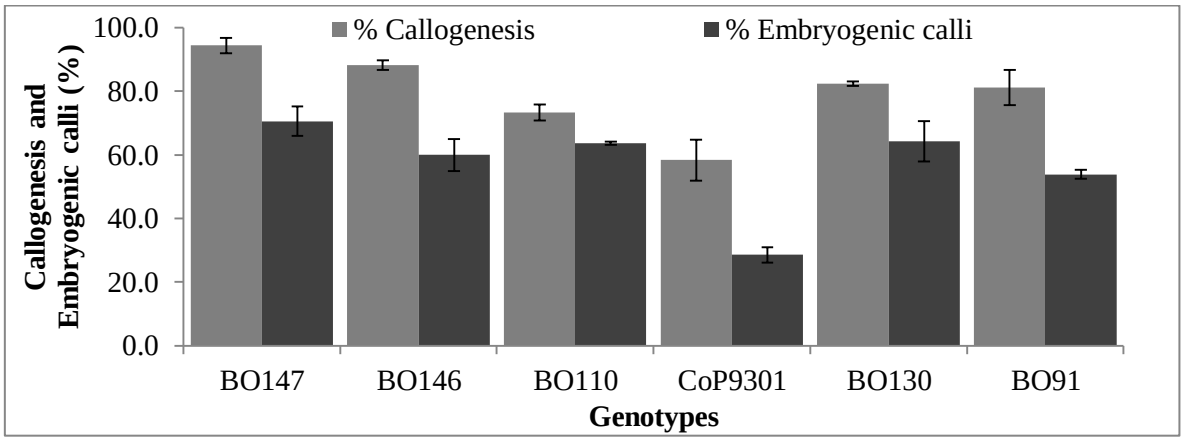

Table 1. Characteristics of developed callus in selected cultivars of sugarcane on MS basal + 2, 4-D (3.0 mgl^{-1}) + 5% sucrose medium

Callus Parameter	Genotypes					
	BO147	BO146	BO110	CoP9301	BO130	BO91
Colour	Greenish cream	Creamish yellow	Yellowish	Brownish	Creamish	Creamish yellow
Nature	Friable with some compact region	Compact	Friable with some compact region	compact	Friable	Friable with some compact region
Growth	++++	+++	+++	+	+++	++
++++ - Excellent, +++ - Good, ++ - moderate, + - Poor						

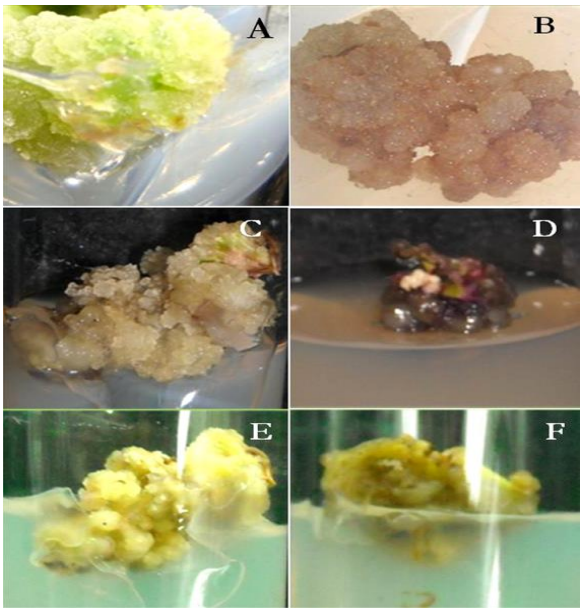

Fig.2.Callogenesis from scaly leaf culture of sugarcane on MS basal + 2, 4-D (3.0 mgl^{-1}) + 5% sucrose:
A. BO147 **B**. BO 146 **C**. BO 110 **D.** CoP 9301
E.BO130 **F.** BO91

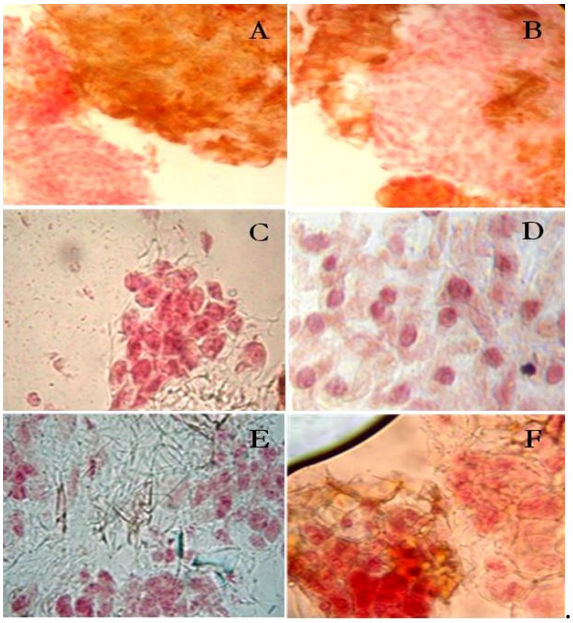

Fig.3. Embryogenic calli of all the six selected cultivars of Sugarcane by Cytological technique showing: (A-B) formation of meristemoid inside the callus (C, D, E & F) meristemoid cells with large vacuolated callus cell

Conclusion

With the present findings it can be concluded that, the best genotype for callogenesis was BO 147, followed BO146, BO130, BO91 and BO110, whereas the poorest was CoP9301. For embryogenic callogenesis the best responded genotype was BO147, followed by BO130, BO110, BO146 and BO91, whereas the poorest was CoP9301. Cytological studies showed formation of meristemoid inside the callus cells with large vacuolated callus cell of genotypes BO 110, CoP 9301, BO130 and BO91.

Thus a differential in vitro response in terms of type and magnitude was observed by different genotypes of sugarcane. This may be due to effect of dominant and additive effect of nuclear and cytoplasm factors as described by earlier workers. Therefore, development of specific callus induction protocol for individual genotypes for mass multiplication and obtaining somaclonal variants is being suggested by this study.

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