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CALLUS INDUCTION AND REGENERATION IN ELITE SUGARCANE CULTIVAR HSF-240

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Abstract

Studies were carried out to establish an efficient system for callus induction and regeneration of sugarcane cultivar HSF-240. Shoot tip with 5-10 mm size was taken as explant for callus induction on MS medium containing different concentrations of 2, 4-D. Among the different concentrations of 2, 4-D used, maximum (80-82%) calli production with 3-4 mm in size were observed on media containing 2, 4-D, for both 2 mg/l and 3 mg/l. Optimum shoots length (8 mm) was obtained on MS medium containing 1.0 mg/l GA_3, 0.5 mg/l Kin and maximum roots (3.6) with maximum length of (3.5 mm) was obtained at 1.0 mg/l IBA.

Introduction

Sugarcane is an important commercial crop in many developing/developed countries. Considering its importance in the agricultural industry, concerted efforts are being made for its improvement using conventional and biotechnological techniques. There are many reports on tissue culture and plant regeneration of sugarcane from different countries. Initial attempts to regenerate plants through *In vitro* technique were made on sugarcane by Nickell (1964) and Heinz & Mee (1969). Callus induction is a very important phenomenon in tissue culture. It is the most important explant for genetic modification. Matsuka *et al*., (2001) initiated calli from the base of young leaves of sugarcane on MS medium containing 2 mg/l 2, 4-D and 3% sucrose. Similarly establishment of callus cultures and regeneration of sugarcane was reported by (Nickel, 1964; Barba & Nickel, 1969). Callus culture of sugarcane have also been successfully established using shoot young leaves and young inflorescence as explants on MS medium containing 2,4-D and coconut milk (Nadar *et al*., 1978; Liu & Chen, 1984; Bhansali & Sing, 1984). Similar response was also reported by Barba *et al*., (1977) and Manan & Amin (1999). Nadar *et al*., (1978); Liu & Chen, 1984; Chen *et al*., (1988) and Lal & Sing, (1991), successfully established callus culture by manipulating 2,4-D concentration in medium. Similarly Kharinarain *et al*., (1996) observed best morphogenic calli production by different genotypes of sugarcane on MS medium supplemented with 3 mg/l 2, 4-D and 5 mg/l diethyldithiocarbamate. However, in Pakistan very few studies were carried out on micropropagation of sugarcane but no study was developed on callus induction from shoot tip which is a base for genetic studies in sugarcane for developing varieties resistant to various diseases and insect pests. In the present study we have optimized conditions for callus induction in sugarcane. This optimized protocol will help in establishing efficient system for the genetic transformation in callus of the important sugarcane varieties for resistance to various stresses such as resistance to diseases and insect pests.

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Materials and Methods

For callus induction *cv*. HSF-240 was selected because it is most cultivated and early maturing variety of sugarcane and has high yield and sugar recovery percentage. The plant materials were provided by Sugar Crops Programme, Crop Sciences Institute and all the experimental work was carried out at Agricultural Biotechnology Programme (ABP), National Agricultural Research Centre (NARC) Islamabad. Surface sterilization was carried out by using 50% clorox for 30 minutes followed by three washings with autoclaved distilled water with each wash for 10 minutes but before that the shoot tips were placed in ethanol for 1 hr and were also treated with antioxidants solution (100 mg/l ascorbic acid + 150 mg/l citric acid) for I hr. Explant of size 5-10 mm was taken from *In vitro* grown established cultures of sugarcane cv. HSF-240. For callus induction 10 different concentrations of 2, 4-dichlorophenoxy acetic acid (i.e 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 3.5, 4.0, 4.5 and 5.0 mg/l) in MS medium (Murashig & Skoog, 1962) along with control (0 mg/l) were used. Two parameters i.e., %age calli induction and size of the calli were studied when the calli were of 20 days old. Various concentrations and combinations of GA_3 + Kin (0+0, 0.5+0, 0.5+0.2, 0.5+0.5, 1.0+0.5 and 1.0+1.0 mg/l) were used for regeneration of shoots while auxin, IBA with 6 different concentrations (0. 0.1, 0.3, 0.5, 1.0 and 1.5 mg/l) were used for rooting of the shoots. Completely Randomized Design (CRD) was used for the statistical analysis by using statistical package MSTAT-C (Anon., 1991).

Results and Discussion

Percentage calli: Percentage calli for shoot tips from *cv*. HSF-240 were highly significantly different ($p<0.01$) for different 2,4-D concentrations (Table 1, Fig. 1). The means for percentage calli ranged from 0% to 82.5%. The highest value (82.5%) was observed for 2 mg/l and 3 mg/l of 2, 4-D while the lowest value (0%) was for that of control (0 mg/l 2, 4-D). Shahid *et al*., (2001) also observed callus formation in 7 species of sugarcane on medium with 2 mg/l 2, 4-D concentration. This strongly supports our results of using 2 mg/l 2,4-D for best callus induction. Similar results were also observed when 2, 4-D was added in modified MS basal medium (Chengalrayan, *et al*., 2001). Fitch & Moore (1990) and Oropeza & Garcia (1996) also inferred that 2-4 mg/l 2, 4-D was best for producing compact calli in sugarcane while Kale *et al*., (2004) initiated callus of sugarcane at 1.0 mg/l 2, 4-D after 5 days with 0.843g fresh weight of the callus. The means for 2.0 mg/l, 2.5 mg/l and 3.0 mg/l 2, 4-D were non-significantly different but were highly significant as compared to all the other means followed by means for 3.5 mg/l 2,4-D (62.5%). Karim *et al*., (2002) also reported 90-100% callus induction at 3.0 mg/l 2, 4-D concentration. These results are very well in line with those of our results. No callus was induced at control as well as 4.5 mg/l and 5.0 mg/l 2, 4-D.

Calli size: The calli size from shoot tips of *cv*. HSF-240 was highly significantly ($p<0.01$) different (Table 1). The calli size ranged from 0 mm for 0 mg/l, 4.5 mg/l and 5.0 mg/l 2, 4-D concentrations to 4.0 mm for 2 mg/l 2, 4-D (Table 1). The means for concentrations 2.0 mg/l, 2.5 mg/l and 3 mg/l 2, 4-D were non-significantly different but were highly significant as compared to all other means. Similarly means for 0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 4 mg/l 2,4-D were also non-significantly different (Table 2). Callus cultures were transferred on MS medium supplemented with 2 mg/l 2, 4-D for maintenance and proliferation, with sub culturing of calli after 10-15 days interval on to fresh medium.

Table 1. Effects of different concentrations of 2, 4-D on callus induction in *cv*. HSF-240.

	df	Mean squares for percentage calli	Mean squares for calli size (mm)
Treatment	10	2426.682**	4.87**
Error	11	19.727	0.102
Total	**21**		
Treatment 2, 4-D (mg/l)		**Means for percentage calli**	**Means for calli size (mm)**
0.0		0.0000 f	0.0000 d
0.5		8.500 ef	1.000 c
1.0		17.50 de	1.000 c
1.5		27.50 c	1.500 c
2.0		82.50 a	4.000 a
2.5		80.00 a	3.500 ab
3.0		82.50 a	3.500 ab
3.5		62.50 b	3.250 b
4.0		25.00 cd	1.000 c
4.5		0.0000 f	0.0000 d
5.0		0.0000 f	0.0000 d

** 'Significant at 1% level' 'Significant at 5% level'

Table 2. Regeneration from calli of *cv*. HSF-240.

Treatments	GA_3 + Kin (mg/l)					
	0 + 0	**0.5 + 0**	**0.5 + 0.2**	**0.5 + 0.5**	**1.0 + 0.5**	**1.0 + 1.0**
Shoot length (mm)	0 D	4.0 B	2.5 C	4.0 B	8.0 A	3.3 BC
Treats IBA (mg/l)	0	0.1	0.3	0.5	1.0	1.5
Root No.	0.0 C	1.6 B	2.0 B	2.0 B	3.6 A	1.6 B
Root length (mm)	0.0 D	1.3 C	1.3 C	1.1 C	3.5 A	2.7 B

Regeneration from calli of cv. HSF-240: Regeneration started with the appearance of green dots on callus within a week on regeneration medium and generally produced normal stem and leaves.

Shoot initiation: The shoot length for different treatments was significantly different ($p<0.01$). The optimum shoot length (8mm) was recorded for treatment containing 1.0 mg/l GA_3 and 0.5 mg/l Kin. Similarly the lowest one (2.5 mm) was recorded for treatment containing 0.5 mg/l GA_3 and 0.2 mg/l Kin, while control did not show any shooting response (Table 2).

Rooting: Root induction was observed in the regeneration medium when plant hormones IBA was added to MS medium. The effect of different concentrations of IBA against root number as well as root length was significantly different ($p<0.01$). The maximum root number (3.6) and root length (3.5 mm) were observed for 1.0 mg/l IBA. Similarly minimum root number (1.6) was achieved for both 0.1 mg/l and 0.5 mg/l IBA. Control showed no response for rooting. Khan *et al*., (1998) observed that use of IBA with 6% sucrose in growth medium induced vigorous root development. The plantlets with well developed shoots and roots were transferred to Jiffy pots having sterilized perlite. After acclimatization the plantlets were first transferred to the earthen pots for hardening and afterward shifted to field. Among different concentrations and combinations for shoot multiplication, best performance was showed on MS medium supplemented with GA_3 1.0 mg/l, Kin 0.5 mg/l and IBA 0.5 mg/l (Table 2). Best rooting was observed on ½ strength MS medium supplemented with 1.0 mg/l IBA (Table 2).

Fig. 1a Callus induction in *cv*.240 at 1.0 mg/l 2, 4-D

Fig. 1b Callus induction in *cv*. HSF-240 at 1.5 mg/l 2, 4-D

Fig. 1c Callus induction in *cv*. HSF-240 at 2.0 mg/l 2, 4-D

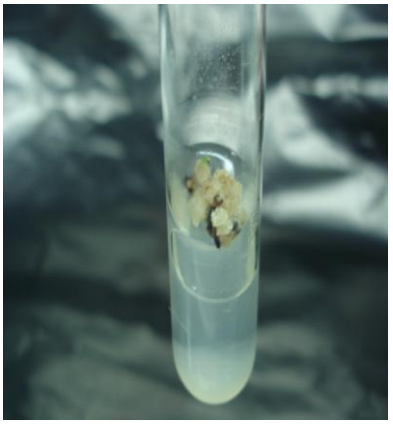

Fig. 1d Callus induction in *cv*.HSF-240 at 2.5 mg/l 2, 4-D

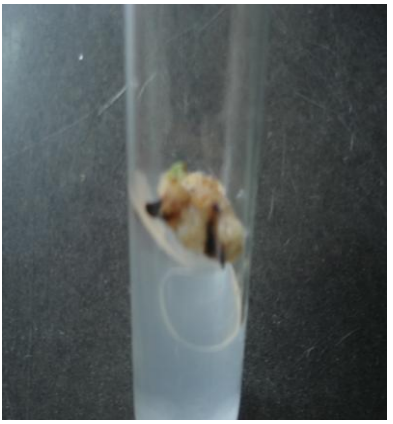

Fig. 1e Callus induction in *cv*.HSF-240 at 3.0 mg/l 2, 4-D

Fig. 1. Callus induction in *cv*.240 at 1.0 mg/l 2, 4-D, Fig. 2 Callus induction in *cv*. HSF-240 at 1.5 mg/l 2, 4-D, Fig. 3 Callus induction in *cv*. HSF-240 at 2.0 mg/l 2, 4-D, Fig. 4 Callus induction in *cv*. HSF-240 at 2.5 mg/l 2, 4-D, Fig. 5 Callus induction in *cv*. HSF-240 at 3.0 mg/l 2, 4-D.

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