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Effect of Growth Hormone and PH Concentration on in Vitro Regeneration of Sugarcane Varieties

Kalpana Sengar[1], R.S. Sengar[2] and Sanjay Kumar Garg[3]

[1]Tissue culture Lab, College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut
[2]Tissue culture Lab, College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut-
[3]Department of Plant Sciences, M.J.P. Roheilkhand University, Bareilly.
E-mail: [1]kalpana.sengar19@gmail.com

Abstract—*Micro propagation is currently the only realistic means of achieving rapid, large- scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in sugarcane. Sugarcane (Saccharum spp. Complex) is the most valuable commercial crop in the world. It holds not only sugarcane and distillery industries but also a key position in the international economy by earning foreign exchanges. The contemporary sugar industry plays many significant roles in relation to food, energy and economic security. Present investigation deals with the effect of growth hormones on in vitro morphogenetic responses of leaf sheath explants of sugarcane varieties CoS 99259, CoS 98259 and CoS 96258. Leaf sheath are suitable explants for shoot regeneration via callus for in vitro micropropagation of sugarcane varieties. In the present study high frequency callus formation was recorded in leaf sheath explants achieved on MS medium containing 3 mg/l 2,4-D while shoot regeneration from the callus was obtained on medium supplemented with BAP, Kinetin or NAA (0.5 mg/l each). The micropropagated shoots of sugarcane were successfully rooted on half strength MS liquid medium containing NAA (5.0 mg/l) and sucrose (50 mg/l). Study for perfect chemical conditions to develop efficient protocol was also done best results were obtained at pH 6.0 in both the varieties.*

1. INTRODUCTION

Commercial Sugarcane belonging to the genus *Saccharum* (Poaceae) is an important industrial crop accounting for nearly 70% of sugar produced world wide. Compared to other major crops efforts to improve Sugarcane are limited and relatively recent, with the first induction of interspecific hybrids about 80 years ago. Production of sufficient quantity of seed material of a new variety of Sugarcane for planting in a vast area generally takes over 10 years if multiplied through conventional methods of seed multiplication. There are also chances of perpetuation of sett-borne diseases. *In vitro* micropropagation technique is emerging as a powerful tool for rapid and large scale production of disease free planting material in a number of crops. Several agro industries and research institutes are now engaged in the micropropagation activities for faster multiplication of newly released and commercially important varieties of Sugarcane (Yadav *et al.* 2004). Sugarcane is the most important sugar crop in the world and accounts for about 70 per cent of the world's total sugar production. In sugarcane, micropropagation is important for rapid multiplication of elite genotypes/clones and for the quick spread of new varieties. Tissue culture of sugar-cane has received considerable research attention because of its economic importance as a cash crop. Micropropogation through tissue culture holds immense potential for mass multiplication and subsequent rejuvenation and quality production. Induction of callus and regeneration of plants using sugar-cane varieties of India were reported elsewhere. However, reports are scarce on shoot tip culture in sugar-cane varieties of India.

Callus Induction and subsequent shoot regeneration in sugarcane were first demonstrated by Heinz and Mee (1986) and Barba and Nickell (1969).Latter, Liu and Chen (1976) demonstrated that sugarcane plant regenerated from callus showed wide variation in chromosome number. Callus cultures of sugarcane have also been successfully established by several investigators (Nadar *et al* . 1978, Bhansali and Singh 1984, Visessuwan *et al* . 1999, Lal 2003) using shoot apices , leaf sheath and young inflorescences as explants on Murashige and skoog (MS) medium supplemented with 2,4-D and coconut milk. Among these explants, young leaf sheath has been widely used as the explants for callus induction about subsequent shoot regeneration in most of the studies. Maximum frequency of callus formation from leaf sheath explants was demonstrated on MS medium containing various concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) (Mannan and Amin1999, Lal 2003, Ramamand *et al* . 2006) Effect of tissue culture explant sources on sugarcane yield component (Hoy *et al* 2003) was also studied. Development of sugarcane *Saccharum* spp. Commercial cross, based on maximum likelihood approach for estimation of linkage and linkage phase (Garcia *et al* 2006).

The present investigation was therefore proposed to study the effect of growth regulators on shoot regeneration from callus

culture of leaf sheath explants of sugarcane varieties CoS 99259 and CoS 96258 and effect of chemical condition such as pH on shoot multiplication .

2. MATERIAL AND METHOD

Fresh tops of sugarcane varieties CoS 99259, CoS 98259 and CoS 96258 were obtained from the field of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. After peeling out the outer leaf sheath the material was wiped with 70 % ethanol. Six to eight cm long spindle segment having growing tips and furled young leaf sheath were then excised out from the tops. For preparation of leaf sheath explants the segments were washed thoroughly under running tap water for 25-30 minutes to remove the dust particles. After washing with tap water, the segments were soaked in 1% (v/v) cetavlon solution for 10-15 minutes followed by through washing with clean water. The material was then rinsed with 70% alcohol for 30-40 seconds followed by washing with sterile distilled water. The material was then surface sterilized with 0.1% (w/v) aqueous solution of mercuric chloride ($HgCl_2$) containing a few drop of Triton X-100 for 8-10 min with continuous shaking. Finally the segments were washed 4-5 times with sterile double distilled water to remove the traces of chemicals. The surface sterilized spindle segments were aseptically dissect out into 1 cm long pieces and spit longitudinally into equal halves with the help of sterile surgical blades and forceps. The leaf sheath explants thus prepared were immediately inoculated on Agar (8.0/l) solidified MS medium (Murashige and Skoog 1962) containing 2,4-D (3.0 mg/l). To investigate the effect of pH on growth and multiplication of shoot cultures, a set of 50 cultures was subcultured separately on media having pH values 5.6, 5.8, 6.0, 6.2 and 6.4. The pH was adjusted with 0.1 N NaOH or 0.1 N HCl before autoclaving. Other physical and chemical parameters were kept constant. A total of 50 cultures were raised for each treatment and the experiments were performed at least thrice.

3. RESULT AND DISCUSSION

3.1 Effect of Growth Regulators on Shoot Regeneration from Leaf Sheath Callus

In order to study the effect of various concentrations and combinations of growth regulators on shoot regeneration from leaf sheath derived callus, an experiment was carried out using MS medium containing 20 g/l sucrose and 8.0 g/l agar. Leaf sheath explants of sugarcane varieties CoS 96258, CoS 98259 and CoS 99259 were inoculated on three different media formulations viz. Murashige and Skoog (MS); Linsmaier and Skoog (LS); and White's medium (WM) with or without 2,4-D to find out the most suitable medium for callus formation. The data presented in Table 4.14 showed that callus formation was achieved at varying degrees on all the three media formulations supplemented with 2,4-D, however, it was the highest on MS followed by LS medium. Callus formation was recorded at a low frequency on WM medium as most of the explants did not respond to callus initiation instead they turned brown and gradually died up. No marked differences were observed in the frequency of callus formation between the explants of both varieties on a particular medium. CoS99259 showed highest callus induction in MS media at 4 mg/l of 2,4-D concentration. (table 1).

On transfer to aforesaid shoot regeneration media, initially numerous pink to light green small protuberances (meristemoids) developed over the surface of callus within 2-3 week of transfer. Often the development of such structures was localized in patches on the callus. These protuberances grew further and developed into green, leafy, minute shoots which attained heights of 1-3 cm within next 3 week

Table 1 : Effect of different nutrient media and 2,4-D concentrations on callus formation from leaf sheath explants of sugarcane varieties.

Nutrient Media	2,4-D (mg/l)	CoS 96258		CoS 99259		CoS 98259	
		Percentage explants showing callus initiation	Callus growth	Percentage explants showing callus initiation	Callus growth	Percentage explants showing callus initiation	Callus growth
MS	0	-	-	-	-	-	-
	1	17.3 ± 1.9	(+)	23.7 ± 2.1	(+)	24.6 ± 2.1	(+)
	2	26.7 ± 3.1	(++)	31.8 ± 4.2	(++)	32.8 ± 4.2	(++)
	3	61.3 ± 4.7	(+++)	56.7 ± 3.3	(+++)	58.7 ± 3.3	(+++)
	4	62.1 ± 5.3	(+++)	54.3 ± 4.3	(+++)	55.3 ± 4.3	(+++)
LS	0	-	-	-	-	-	-
	1	12.3 ± 1.4	(+)	15.7 ± 2.1	(+)	16.7 ± 2.1	(+)
	2	24.3 ± 1.9	(+)	27.3 ± 3.1	(+)	28.3 ± 3.1	(+)
	3	45.7 ± 4.7	(++)	47.3 ± 4.3	(++)	48.3 ± 4.3	(++)
	4	37.5 ± 4.1	(++)	48.4 ± 4.7	(++)	49.4 ± 4.7	(++)
WM	0	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	3.6 ± 0.6	(+)	5.1 ± 0.8	(+)	6.1 ± 0.8	(+)
	3	11.3 ± 1.6	(+)	14.3 ± 1.7	(+)	15.3 ± 1.7	(+)
	4	12.6 ± 1.3	(+)	13.7 ± 1.3	(+)	14.7 ± 1.3	(+)

Callus growth (+) poor; (++) moderate (+++) good

The growing shoots were carefully picked out aseptically and transferred to MS liquid medium containing BAP, Kn and

NAA (0.5 mg/l each) which was adjudged as the most appropriate combination of growth regulators in earlier experiment. On transfer to this medium, the shoots grew rapidly and developed numerous side shoots (tillers) resulting into the formation of shoot clumps (fig. 1, 2).

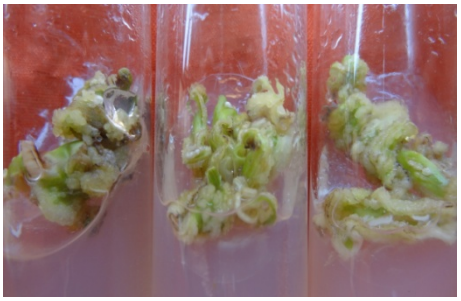

Fig. 1: Callus formation on leaf sheath

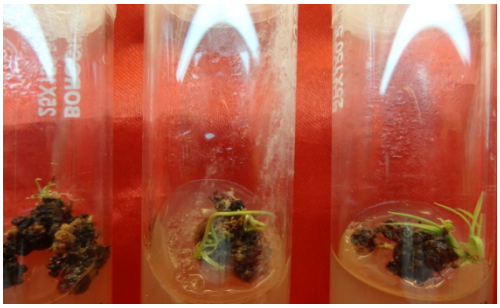

Fig. 2: Shoot initiation from callus

3.2 Effect of pH

The investigation suggested that environmental effect plays a significant role in the stipulation of *in vitro* shoot multiplication. Various response were observed in both the selected varieties in terms of temperature, pH and photoperiod effect. An experiment was carried out with multiplication medium having various pH values (5.4 to 6.6). The results presented in table-2 shows that the rate of multiplication and shoot growth were highly influenced by the pH of medium. The best results were obtained at pH 6.0 in all three varieties followed by 6.2 pH of the medium. In variety CoS 96258 at 6.0 pH it was 61.5 ± 4.7 and lowest 33.2 ± 2.2 at 5.4 pH, while in case of variety CoS 99259 at 6.0 pH, it was found 68.3 ± 4.9 and lowest 36.5 ± 1.7 at 5.4 pH. The results conclude that when the pH of the medium increases up to 6.0 the number of shoots per cultures increased accordingly. A further increase in pH of medium significantly reduces the number of shoots per culture. As regards growth of the shoots, good results were observed between both the varieties at 6.0 pH, The results also suggested that the pH of medium has considerable impact on rate of shoot multiplication and shoot growth. Lal 2004, Wagih *et al.* 2004). The responses regarding multiplication of shoot cultures were also influenced due to pH of the medium. It is reported that multiplication rate in terms of number of shoots per cultures was higher at pH 6.0 and pH 54 & 6.6 gave poor responses. The pH of medium possibly plays significant role in regulating the cellular metabolic activities through its effect on related enzymes further affects the cellular growth and differentiation. This argument is also supported by the fact that each of the metabolic enzymes has pH optima at which it is most active. Conclusively, the results show a considerable impact of pH on growth and multiplication of shoot cultures *in vitro*. This study highlights the need to investigate the effects of environmental conditions when developing efficient micro-propagation protocols, especially for commercial purposes.

Table 2: Effect of pH on shoot regeneration from leaf – sheath callus of sugarcane varieties.

pH of the medium	CoS 96258		CoS 99259		CoS 98259	
	Regeneration (%)	No. of shoots / culture	Regeneration (%)	No. of shoots / culture	Regeneration (%)	No. of shoots / culture
5.4	33.2 ± 2.2	2.1±0.7	36.5 ± 1.7	4.3±0.6	35.5 ± 2.7	5.1±0.6
5.6	35.4 ± 2.1	5.2±0.7	38.5 ± 2.7	6.3±0.6	39.9 ± 2.7	4.3±0.6
5.8	42.3 ± 3.3	6.6±0.6	44.6 ± 3.6	8.4±0.8	42.6 ± 3.6	7.9±0.8
6.0	61.5 ± 4.7	16.3± 1.6	68.3 ± 4.9	18.7± 1.7	66.3 ± 4.9	18.7± 1.7
6.2	59.4 ± 3.9	15.4± 0.7	61.5 ± 4.1	17.9± 1.4	61.5 ± 4.1	16.4± 1.4
6.4	43.1 ± 3.2	11.8± 0.6	46.3 ± 3.5	12.4± 0.3	48.3 ± 3.5	12.4± 0.3
6.6	41.3 ± 3.3	6.6±0.6	42.6 ± 3.6	7.4±0.8	40.6 ± 3.6	6.9±0.8

4. ACKNOWLEDGEMENTS

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