Indian Journal of Sugarcane Technology **28(02)**: 50-52, December 2013

In vitro micropropagation of sugarcane: optimization of responses through hormonal manipulation

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ABSTRACT

An experiment was carried out to optimize *in vitro* multiplication of shoot cultures and rooting of shoots in sugarcane varieties 'CoS 07250' and 'CoS 08272'. About 1.0 cm long shoot-tips comprising apical meristem and 1-2 leaf primordia were excised, and after surface sterilization cultured onto semisolid MS medium supplemented with different concentrations/ combinations of BAP, kinetin and NAA. MS medium supplemented with BAP, Kinetin and NAA (0.5 mg/l each) was the most suitable for producing optimum number of shoots in both the varieties. For rooting, well-grown shoot cultures were separated in smaller groups containing 4-6 shoots and transferred onto half-strength MS liquid medium containing various concentrations of sucrose (30, 50 or 70 g/l) and NAA (3.0, 5.0 or 10.0 mg/l). The maximum (92%) shoot cultures developed vigorous root system on half-strength MS liquid medium containing 50 g/l sucrose and 5.0 mg/l NAA. The rooted shoots were washed, hardened for 45 days in the green house and thereafter transplanted in the field with more than 96% survival.

Key words*: Sugarcane, micropropagation, growth regulators.*

The success of sugarcane production programme depends on the rate of multiplication of new varieties as it not only helps in rapid spread but also in quick adoption of new varieties over a larger area. In sugarcane, the production of sufficient quantity of seed material of a new variety takes 8-10 years through conventional methods of seed multiplication, and by the time the varieties start deteriorating. There are also chances of perpetuation of sett-borne diseases. *In vitro* micropropagation techniques are now emerging as a powerful tool to overcome such problems (Tiwari *et al*., 2010). Several investigators have suggested different protocols during the past three decades for *in vitro* micropropagation of sugarcane varieties (Hendre *et al.* 1975; Hendre *et al.* 1983; Sauvaire and Galzy 1978, Lee 1987, Lal and Singh 1994, Shukla *et al.* 1994). The previous reports indicate that the hormonal requirements for *in vitro* morphogenetic responses vary due to sugarcane genotype. Thus present study was undertaken to optimize the hormonal requirement for enhanced production of plantlets of sugarcane varieties 'CoS 07250' and 'CoS 08272' through *in vitro* micropropagation technique.

MATERIALS AND METHODS

Approximately 8 cm long spindle segments were dissected out from the freshly collected tops of sugarcane varieties 'CoS 07250' and 'CoS 08272' growing at the research farm of U.P. Council of Sugarcane Research, Shahjahanpur. These segments were washed under running tap water for ~20 min followed by 5 min rinse with 1% detergent solution. After several washing with water, the segments were finally surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 10 min followed by several washings with sterile distilled water. About 1.0 cm long shoot-tips comprising apical meristem and 1-2 leaf primordia were excised from the sterilized segments and immediately inoculated onto semisolid MS medium (Murashige and Skoog 1962) supplemented with 0.5 mg/l each of BAP and Kinetin.

Once the shoot cultures were established, they were sub-cultured on MS liquid multiplication medium containing BAP and Kinetin alone or in combination with NAA and GA_3 (0.5 mg/l each) for shoot multiplication. For rooting, the regenerated shoots clumps were separated in smaller groups containing 2-4 shoots and transferred on half-strength MS liquid medium containing NAA (3.0, 5.0 or 7.0 mg/l) in combination with different concentrations of sucrose (30, 50 or 70 g/l). All the cultures were maintained in growth room at 25±2ºC under 16 h illumination of cool white fluorescent tubes at an intensity of 4000 lux. A total of 24 cultures were raised for each treatment and the experiments were replicated thrice.

RESULTS AND DISCUSSION

Initially, the shoot tip explants, inoculated on MS shoot initiation medium, secreted enormous amount of phenolic substances causing browning of the medium. This problem was overcome by transferring the explants onto the fresh

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Table 1 Effect of growth regulators on multiplication and growth of shoot cultures in sugarcane varieties 'CoS 07250' and 'CoS 08272'

Growth regulators in MS medium (mg/l)	Number of shoots per culture*		Shoot growth	
	'CoS 07250'	'CoS 08272'	'CoS 07250'	'CoS 08272'
BAP 0.5	7.5±0.6	9.0±0.7	Poor	Poor
Kin 0.5	6.6±0.7	8.5±0.5	Poor	Poor
BAP 0.5 + Kin 0.5	14.7±1.3	19.7±1.5	Moderate	Moderate
BAP 0.5 + Kin 0.5 + NAA 0.5	22.8±1.9	27.3±2.1	Good	Good
BAP 0.5 + Kin 0.5 + NAA 0.5 + GA_3 0.5	19.3±1.7	22.3±1.4	Good	Good

*Shoots <1.0 cm in length were not considered.

Table 2 Effect of different concentrations of NAA and sucrose on rooting of shoots derived from shoot tip explants of sugarcane varieties 'CoS 07250' and 'CoS 08272'

Half-strength MS medium		'CoS 07250'			'CoS 08272'		
NAA (mg/l)	Sucrose (g/l)	Rooting response (%)	Number of roots per plant	Root vigour	Rooting response (%)	Number of roots per plant	Root vigour
3.0	30	31.3±2.7	3.4±0.3	Poor	33.3±3.1	3.5±0.3	Poor
5.0		46.6±4.8	4.1±0.3	Moderate	49.6±5.2	4.2±0.4	Moderate
7.0		44.2±4.9	4.3±0.4	Moderate	52.5±3.3	4.4±0.4	Moderate
3.0	50	64.8±6.1	4.6±0.4	Poor	65.3±5.5	4.2±0.3	Poor
5.0		84.4±5.2	5.8±0.5	Good	88.5±6.3	5.3±0.5	Good
7.0		72.6±5.3	4.3±0.4	Good	74.8±5.7	4.5±0.4	Good
3.0	70	56.8±4.2	4.1±0.3	Poor	59.9±4.4	3.6±0.3	Poor
5.0		72.2±5.6	4.0±0.4	Moderate	75.1±4.8	4.3±0.4	Moderate
7.0		59.2±4.3	4.3±0.4	Moderate	64.7±4.6	4.1±0.3	Moderate

(Mean± SD), *Roots < 0.5 cm in length were not considered.

medium at 2-3 days interval for initial 10 days. Swelling of bud primordia was observed within 3-7 days of inoculation. The bud primordia grew further and developed 1 shoot within 3 week in most of the responding cultures. The cultures were then transferred onto MS liquid medium of the same composition for establishment. Several side shoots developed within next 3-4 weeks in 50% and 60% explants in variety 'CoS 07250' and 'CoS 08272', respectively giving rise to shoot clumps.

The shoot clumps (established cultures) were separated in groups of 2-4 shoots and transferred on MS multiplication medium containing various growth regulators alone or in combination (Table 1). The results showed that the maximum of 27.3 shoots per culture could be produced in MS medium containing 0.5 mg/l each of BAP, Kinetin and NAA in variety 'CoS 08272', which was significantly higher than all other treatments. Addition of GA_3 (0.5 mg/l) to the above medium showed a marginal reduction in number of shoots in both the varieties. It is evident from Table 1 that BAP or Kinetin alone induced about 6.6 to 9.0 shoots per culture, however, a significant increase in number of shoots was observed when both the cytokinins were used simultaneously. Use of NAA (0.5 mg/l) along with 0.5 mg/l each of BAP and Kinetin further increased the number of shoots per culture. Production of enhanced number of shoots with the addition of an auxin to a cytokinin containing medium has been reported earlier (Pawar *et al.* 2002). The shoots produced on medium containing NAA and GA_3 along with the cytokinins showed active growth and appeared to be vigorous, whereas, those produced on medium devoid of NAA and GA_3 showed moderate to poor growth. On comparison, it was found that variety 'CoS 08272' showed better morphogenetic responses regarding establishment of shoot cultures, number of shoots per culture and shoot growth than the variety 'CoS 07250'.

The results presented in Table 2 revealed that an increase in concentration of NAA from 3.0 to 5.0 mg/l, irrespective of the concentration of sucrose, significantly enhanced the frequency of rooting in both the varieties. Similarly, an increase in the concentration of sucrose from 30 to 50 g/l significantly

improved the frequency of rooting at a particular concentration of NAA. However, when the concentration of sucrose was further raised to 70 g/l, the rooting frequency declined in both the varieties. The maximum rooting could be recorded to be 84.4% in variety 'CoS 07250' and 88.5% in 'CoS 08272' on medium containing NAA (5.0 mg/l) and sucrose (50 g/l) which were significantly higher than other treatments. These results indicated the individual role of NAA and sucrose concentrations in enhancing the frequency of rooting in micropropagated shoots of sugarcane.

As the number of roots per shoot is concerned, the maximum 5.8 roots per shoot was obtained in the variety 'CoS 07250' in presence of NAA (5.0 mg/l) and sucrose (50 g/l), whereas, it ranged from 3.4 to 4.6 roots per shoot in other treatments. Similarly, the highest number of roots (5.3) could be induced in variety 'CoS 08272' on the same medium. Roots produced on media containing NAA (5.0 or 7.0 mg/l) in combination with sucrose (50 g/l) were vigorous in growth and healthy in appearance, whereas, those produced under other treatments showed poor development.

CONCLUSION

It was found that MS medium supplemented with 0.5 mg/l each of BAP, Kinetin and NAA was the most effective for shoot multiplication and half-strength MS medium containing 50 g/l of sucrose in combination with 5.0 mg/l NAA was the most suitable for induction of rooting in micropropagated shoots of sugarcane varieties.

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