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IN VITRO REGENERATION OF *SACCHARUM OFFICINARUM* VAR. CO 92005 USING SHOOT TIP EXPLANT

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ABSTRACT
Micropropagation is a widely implemented technique for production of a large number of plantlets in a very short period of time and in a trifling space of a culture room. Shoot tips and meristem culture techniques offer an opportunity for virus free plants production. In the present study, shoot tip culture technique was used for the production of plantlets of *Saccharum officinarum* (var. Co 92005). Shoot tips of size 0.5 cm dissected out from actively growing twigs of 6-8 month old plants, were cultured on MS basal medium supplemented with different concentrations of BAP and coconut milk. MS medium with 0.5 mg/l BAP and 20 per cent coconut milk was found to be best for culture initiation and establishment with 100 per cent response and maximum elongation of shoots. The same medium was found better for multiple shoot formation with 100 per cent response and maximum shoot multiplication. Maximum rooting response was observed on MS medium with 2.5 mg/l IBA with 100 per cent response with an average number of roots 2.93 roots/shoot and average root length 2.5 cm within two weeks. The rooted plantlets were transferred to pots filled with coco peat and were hardened under a polytunnel with 90 per cent survival after 15 days.

KEYWORDS: Micropropagation, *Saccharum officinarum* (var. Co 92005), MS Medium, Coconut Milk.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) belonging to family Gramineae (Poaceae) is one of the major cash crop grown in India. Main product of sugarcane is sugar and its juice is used for the preparation of various products like jaggery, molasses, bagasse and ethanol. Conventionally sugarcane propagation is carried out by stem cuttings using three budded setts. Disease-ridden planting materials can pass diseases like grassy shoot, red rot, ratoon stunting, leaf scald, etc. to succeeding crops. This leads to financial loss due to a reduction in cane yield and sucrose recovery. Plant tissue culture offers a way to overcome these issues and to produce disease-free planting material. Technique of plant tissue culture, 'micropropagation' is the most successful and tranquil way to produce ample amount of pathogen free and vigorous planting material. Micropropagation defined as, 'tremendous increase in the number of shoots available for rooting results from the proliferation of the lateral buds or adventitious shoots or the differentiation of the shoots'. This method has several advantages over the conventional method of propagation. Meristematic tissues are mainly used in micropropagation to produce disease free planting material. According to Hendre *et al*. (1983), about 0.2 million plants can be produced from single sugarcane shoot tip within six months. According to Dhumale *et al*. (1994), single sugarcane shoot tips of size 2-3 mm can give significant shoot regeneration. Millions of clonally uniform plants can be produced within a year from single explant by using micropropagation technique. MS Basal medium supplemented with benzylamine purine (BAP) and kinetin (Kn) effectively used for rapid shoot multiplication (Ali and Afghan, 2001; Singh *et al*., 2006; Mekonnen *et al*., 2014). According to Schenk and Hildebrandt (1972), sugarcane requires a high concentration of auxin for rooting. Roots can be regenerated from in vitro regenerated shoots on half MS medium supplemented with NAA, IBA and IAA (Baksha *et al*., 2002; Khan *et al*., 2008). For each new variety and clone, efficient in vitro regeneration protocol is essential to guarantee the rapid shoot initiation, shoot multiplication, elongation and root induction (Cheema and Hussai, 2004).

The sugarcane variety Co 92005 recorded more cane yield, higher sugar yield, and more jaggery recovery compared to other varieties grown in Maharashtra. Its jaggery quality is better and fetches a higher market price. This variety is commercially recommended for jaggery production in Maharashtra. The present study was undertaken to study *in vitro* regeneration in *S. officinarum* var. Co 92005 using shoot tip explant, in order to determine an effective protocol for rapid plantlets multiplication. This paper presented the first time report of such study in *S. officinarum* var. Co 92005.

MATERIALS & METHODS

Collection of plant material

Healthy, fresh 6-8 months old sugarcane plant twigs were collected from fields of Vidya Pratishthan's VSBT College of Agricultural Biotechnology, Baramati, Maharashtra. The twigs were excised in the morning and brought to the laboratory in glass bottles containing distilled water in order to prevent browning.

Media preparation

For the establishment of explant, MS medium supplemented with 3 per cent sucrose, 10 per cent (v/v) coconut water, 0.8 percent agar and different concentrations (0.1, 0.3, 0.5, 1.0, 2.0 mg/l) of BAP were prepared. pH of medium was adjusted to 5.75. In each tubes (25×150 ml) 20 ml media was poured and sterilised by autoclaving at 121 temperature and 15 psi pressure for 20 min.

Preparation of explants

Explants were washed thoroughly under running tap water and treated with fungicide (Bavistin) (1 per cent v/v) for 10 min. to reduce the fungal contaminants. The explants were then treated with a solution of the Savlon (1 per cent v/v) for 10 min. and 70 per cent ethanol for 30 sec. under aseptic conditions to reduce bacterial contamination. Then surface sterilized with $HgCl_2$ (0.1 per cent w/v) for 10 min. under aseptic conditions to reduce remaining contamination. The explants were washed three times with sterile distilled water and then transferred in antioxidant solution of ascorbic acid (1 per cent v/v) to reduce browning of explants. After surface sterilization explant were dissected to get shoot tip of size about 0.5 cm under aseptic conditions.

Establishment, subculturing and multiplication of shoots

Excised shoot tips were placed aseptically on medium supplemented with different concentrations of BAP. All explants were incubated in culture room at 25 ± 2 and under 2000 lux intensity of white cool fluorescent lamp with 16:8 photoperiods. Cultures were observed in regular day interval. Regenerated shoots then subcultured on fresh medium for its better growth and multiple shoots production. MS basal media supplemented with different concentrations of BAP (0.1, 0.3, 0.5, 1.0, 2.0 mg/l) and 20 per cent (v/v) coconut water, 3 per cent sucrose and 0.8 per cent agar were used for multiplication of regenerated shoots.

Rooting and primary hardening

Multiplied shoots were separated as single shoots for root initiation. Single shoots were transferred aseptically on medium containing full strength MS basal media and half strength MS basal media with different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) of IBA for root initiation. Culture bottles were incubated at 25±2 and 2000 lux intensity of white cool fluorescent lamp with 16:8 photoperiod. After every three days observations were taken for root initiation and root elongation. Primary hardening was carried out in soil, soilrite, cocopeat and vermicompost separately with ten replicates in a polyhouse under controlled temperature and humidity.

RESULTS & DISCUSSION

Surface sterilization of explant with 1% Bavistin for 10 min., 1% savlon for 10 min., 70 % ethanol for 30 sec. and 0.1 % $HgCl_2$ for 10 min. had shown improved culture establishment without any contamination. The shoots became green and callusing at the cut end started within 3-4 days after inoculation. After 8-9 days of inoculation shoots were developed in all tubes. Regenerated shoots were subcultured after 8 days of inoculation on fresh medium to diminish browning of explant at the base. Per cent response of shoots regeneration was 100 per cent on all the concentrations of BAP tested. However, the best elongation of shoot (2.05 ± 0.07 cm) was observed on the medium supplemented with 0.5 mg/l BAP (Table 1).

TABLE 1: Effect of different concentrations of BAP on establishment of explant and on its length

Concentration of BAP (mg/l)	Number of shoots inoculated/bottle	Number of shoots established/bottle	% Response	Average length of shoots (cm)
0.1	6	6	100	1.40 ± 0.08
0.3	6	6	100	1.40 ± 0.10
0.5	6	6	100	2.05 ± 0.07
1.0	6	6	100	1.06 ± 0.13
2.0	6	6	100	1.60 ± 0.13

For the multiplication of regenerated shoots, MS basal medium supplemented with different concentrations of BAP were used. Among the media compositions tested, MS medium supplemented with 0.5 mg/l had shown maximum rate of multiplication (30 ± 0.31 shoots/culture bottle) with an average length (3.4 ± 0.05 cm) of shoots (Table 2).

TABLE 2: Effect different concentrations of BAP on shoot multiplication

Concentration of BAP (mg/l)	Number of explants/bottle	Average number of multiple shoots/bottle	Average number of shoots	Average length of shoots (cm)
0.1	5	16 ± 0.24	3.2 ± 0.24	1.0 ± 0.06
0.3	5	14 ± 0.28	2.8 ± 0.28	1.5 ± 0.10
0.5	5	30 ± 0.31	6.0 ± 0.31	3.4 ± 0.05
1	5	12 ± 0.14	2.4 ± 0.14	2.1 ± 0.09
2	5	10 ± 0.54	2.0 ± 0.54	1.8 ± 0.12

For root induction, the shoots were inoculated on half and full strength MS basal medium supplemented with various concentrations of IBA. It was found that the full strength medium was superior to half strength MS basal medium for root induction. The root initiation was observed after 10 days of inoculations on rooting medium. The maximum rooting was observed on full strength MS basal medium supplemented with 2.5 mg/l IBA with 100 per cent response of the explants with an average 2.93 ± 0.11 numbers of roots (Table 3).

TABLE 3: Effect of different concentrations of IBA on root initiation

Concentration of IBA (mg/l)	Full strength medium		Half strength medium	
	% Response	Average number of roots/explant	% Response	Average number of roots/explant
0.5	0	0	0	0
1.0	46.00	1.42 ± 0.02	40.00	1.25 ± 0.07
1.5	66.66	1.00 ± 0.07	36.00	1.45 ± 0.05
2.0	80.00	1.87 ± 0.08	86.00	1.57 ± 0.04
2.5	100.00	2.93 ± 0.11	66.66	1.20 ± 0.06

In primary hardening after 10 days of transplanting in polytunnel, shoots were green and growth was observed. The survival of plantlets obtained was 90 per cent in cocopeat as compared to soil (32 %), soilrite (46 %), and vermicompost

DISCUSSION

For the establishment of meristem culture, MS medium supplemented with BAP was similar to results of Biradar *et al.* (2009) who determined maximum frequency of establishment (72 %) in sugarcane var. CoC-671was with 2.0 mg/l BAP. Dhumale *et al*. (1994) reported that the MS basal medium supplemented with BAP (3 mg/l) and NAA (1 mg/l) had shown better shoot development. The remarks propose that the use of cytokinin and its concentration changes according to the genotype. MS medium supplemented with 0.1 mg/l to 2.0 mg /l BAP can be used for the establishment of the sugarcane shoot tips as it has given 100 per cent establishment. Nevertheless, for higher elongation of shoots, moderate concentration of BAP is enough. MS medium supplemented with 0.5 mg/l BAP had shown maximum rate of multiplication similar to results obtained by Biradar *et al.* (2009) who reported that MS medium containing 1.0 mg/l of BAP had given maximum multiplication of shoots with desirable quality; shoots were well-grown, easily separable and healthy plantlets. Also 0.5-1 mg/l BAP is good for shoot multiplication of sugarcane (Alam *et al.*, 1995). Sugarcane (var. CO-86032) was able to form maximum shoots on MS medium supplemented with 0.3 mg/l BAP (Godheja *et al*., 2014). For normal development of the shoot from the bud-meristem, requires low levels of the growth regulators (Sreenivasan and Jalaja, 1983).

The maximum rooting was observed on full strength MS basal medium supplemented with 2.5 mg/l IBA as reported by Khan *et al.* (2008) that the IBA is essential for rooting in sugarcane. Biradar *et al.* (2009) have observed 80 per cent rooting of sugarcane cultivar CoC- 671 on medium supplemented with 2 mg/l NAA, whereas 70 per cent rooting was obtained on medium supplemented with 3 mg/l NAA. Many researchers also reported that 5 mg/l NAA was good for rooting (Larkin, 1982; Shukla *et al*., 1994; Alam *et al*., 1995; Islam *et al*., 1996) and more than 5 mg/l NAA inhibits rooting. Similar findings were observed in present study.

In present study it was observed that, the only 6-7 months old plants give better establishment of shoots within a period of one week whereas, explants of younger mother plants (3-4 months) had took more than two weeks. Low concentration (0.5mg/l) of cytokinine is effective for the shoot growth and multiplication of shoots. Full strength MS basal had shown better root initiation as compared to half strength. Cocopeat is the best suitable medium as compared to soil and soilrite for primary hardening of sugarcane.

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