



## MICROPROPAGATION OF FOUR SUGARCANE VARIETIES USING SHOOT TIP, LEAF ROLL AND APICAL MERISTEM EXPLANTS

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ABSTRACT

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Four sugarcane varieties (87A298, Co86032, CoT8201 and 2003V46) and three explants (shoot tip, leaf roll and apical meristem) were used for micropropagation and observations were made on callus, shoot and root induction. Among all four varieties 87A298 responded well for callus, shoot and root induction and a opposite performance was observed for CoT8201.

**KEYWORDS:** Sugarcane, micropropagation

### INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important agricultural cash crop in tropical and sub tropical region of the world and is the major source of sugar, ethanol, biogas, manure, production of electricity and paper. It is the only member of the family Gramineae belong to genus *Saccharum* in which *in vitro* propagation are standardized and commercially viable. In sugarcane, production of sufficient quantity of seed material of a new variety takes several years (8-10 years) if multiplied through conventional methods of seed multiplication, by the time the varieties start deterioration in yield potential. There are also chances of perpetuation of sett-borne diseases. *In vitro* micropropagation technique is becoming useful in rapid

multiplication of virus free materials through apical meristem culture (Hendre *et al.*, 1983) and callus culture (Ali *et al.*, 2007). Several investigators have suggested various protocols during the past three decades for *in vitro* micropropagation of sugarcane varieties (Jadhav *et al.*, 2001; Pawar *et al.*, 2002; Sengar *et al.*, 2012; Chaudhary *et al.*, 2013).

The present study was therefore undertaken to study *in vitro* micropropagation of four sugarcane varieties (87A298, Co86032, CoT8201 and 2003V46) using three explants (shoot tip, leaf roll and apical meristem)

### MATERIALS AND METHODS

#### Explant source

Healthy tops of 87A298, Co86032, CoT8201 and 2003V46 were collected from the field grown plants of sugarcane (*Saccharum officinarum* L.) maintained at Agricultural Research Station, Perumallapalle, Tirupati.

#### Surface sterilization of explants

Actively growing points of sugarcane top, taken as explants from 8-12 months old sugarcane cultivar. Outer whorls of mature leaves were removed till yellowish white covering around apical meristems appear. After removing outer whorls of leaves, the tops are sized to 10 cm length by cutting off at the two ends. These were then washed with sterile distilled water thrice and then treated with 3 per cent (w/v) sodium hypochlorite (HgCl<sub>2</sub>) solution for about 10 minutes in laminar air flow chamber and washed out thrice with sterile distilled water. The explants (apical shoots) are picked carefully with sterile forceps and placed in a sterile petri dish. Using a fine forceps and scalpel, the outer leaf sheaths are removed one by one without exerting pressure on the internal tissues. The process is repeated until the apical dome with two or three leaf primordia is exposed.

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ter excising the apex, the explants are transferred immediately on a filter paper support immersed in MS medium supplemented with different concentrations of growth regulators. All the above operations were performed under aseptic conditions in laminar airflow cabinet.

### **Culture medium and condition**

All the three explants of four varieties were inoculated on to sterilized semisolid basal MS medium (Murashige & Skoog's, 1962) supplemented with different concentrations and combinations of different plant growth regulators.

### **Callus induction**

MS medium supplemented with 2, 4-D (3.0 mg l<sup>-1</sup>) was used for callus induction.

### **Shoot regeneration medium**

White friable calli were cultured on MS medium supplemented with BAP (3 mg l<sup>-1</sup>), IAA (2 mg l<sup>-1</sup>) and Kinetin (2 mg l<sup>-1</sup>) were used for shoot regeneration. It was sub-cultured every 7 days of incubation.

### **Rooting medium**

For root induction, elongated micro shoots measuring about 5-6 cm in length were excised from culture tube and transferred to half strength MS medium supplemented with NAA (2 mg l<sup>-1</sup>) and sucrose (30 g l<sup>-1</sup>).

### **Environmental condition**

In medium, the pH was adjusted in the direction of 5.8 and autoclaved for 15-20 minutes at 121°C and at 15 lbs psi. The cultures were incubated with 16 hour of light and 8 hour of dark (fluorescent tubes are used as a light source), with artificial illumination of 2000-3000 lux by placing the cultures at 25-30cm below the fluorescent light and maintained the temperature at 25 ± 2°C. Sub cultures were done for every 2-3 weeks according to the need of the experiment.

### **Acclimatization and transfer of plantlets to soil**

Plantlets with well developed roots were removed from the culture medium. Washed the roots gently under running tap water and were transferred to poly bags for

hardening which contain autoclaved farm yard manure, soil and sand (1:1:1). The harden plantlets were covered with porous polythene sheets for maintaining high humidity and were kept under shade in a net house for further growth and development. After 30 days the plantlets were transplanted in to the soil in field condition.

### **Statistical analysis**

Completely Randomized Design (CBD) was set up in an experiment with three replications. In each replication, 10 explants were used per treatment. Observations were recorded in terms of number of explants induced callus, number of days taken for callus induction, callus induction percentage, time taken for shoot induction, number of days for shoot initiation, shoot regeneration frequency, number of shoots per explant, average shoot length (cm), time taken for root induction, root induction frequency, number of roots produced per shoot, average root length (cm), number of days for acclimatization and survival percentage.

## **RESULTS AND DISCUSSION**

Callus induction frequency in the leaf rolls of Co86032 and 2003V46 was higher (76.66% and 63.55%) and took more number of days (6.9 and 5.8) for callus induction than apical meristem cultures (Table 1.) However reverse was true with respect to 87A298 and CoT8201. These results were consistent with the report of Kaur and Kapoor (2016), Dinesh *et al.*, (2015), Abu *et al.* (2014) and Tiwari *et al.*, (2013) who studied in vitro micropropagation in various sugarcane varieties and found that 3 mg l<sup>-1</sup> of 2, 4-D was the best concentration for callus induction. Maximum of 70.7 ± 8.1 per cent explants showed callus initiation on MS medium containing 3.0 mg l<sup>-1</sup> of 2, 4-D (Goel *et al.*, 2010). Dash *et al.*, (2011) reported the highest callus induction frequency of 95 per cent from shoot tip at 3 mg l<sup>-1</sup> of 2, 4-D.

In general shoot regeneration frequency was higher in shoot tip explants of the varieties with an exception to Co86032, where in leaf roll explant exhibited higher shoot regeneration frequency (76.6%) than the other explants (Table 2.). The time taken for shoot initiation was more in shoot tip derived explants than the other explants of respective varieties except Co86032 apical meristem (41.7 days). Leaf roll explants of all the varieties took less time for shoot initiation. Similarly leaf roll explants

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produced more number of shoots per explant except CoT8201 shoot tip (13.6). Garcia *et al.*, (2007) observed shoot regeneration after 30 days of inoculation. Biradar *et al.* (2009) and Godheja *et al.*, (2014) reported the shoot induction within 7-10 days of culture.

Yadav *et al.*, (2013) recorded maximum of  $53.3 \pm 2.7$  per cent and  $51.1 \pm 3.2$  per cent shoot initiation was recorded in CoSe01235 and CoS99259, respectively for shoot tip explant and  $35.2 \pm 3.4$  per cent and  $34.3 \pm 3.9$  per cent for meristem culture on MS medium containing BAP and Kinetin (0.5 mg l<sup>-1</sup> each). Tripathi and Lal (2013) observed shoot regeneration of 80 per cent, 65 per cent and 40 per cent in shoot tip, leaf roll and bud explants respectively. Sughra *et al.*, (2014) observed average number of 6.50, 4.70 and 3.80 shoots per explant in BL-4, Thatta-10 and Larkana-2001 respectively. Yadav *et al.*, (2013) obtained 5.8 cm and 5.6 cm shoot length in CoSe 01235 and CoS 99259 respectively.

Apical meristem of 87A298 and Co86032 took more time (13.1 days and 12.4 days) for root induction with high root induction frequency (87.50% and 69.32%) (Table3). Where as in shoot tip explant of CoT8201 and leaf roll explant of 2003V46 recorded higher root induction frequency (54.17% and 85.06%) than the other explants of the respective varieties and took more time for root induction (9.3 days and 14.1 days). These results were supported by Sood *et al.*, (2000) reported root induction in 10 -15 days on MS medium + 7 mg l<sup>-1</sup> NAA. On contrary, Sughra *et al.* (2014) observed root induction of 9 days, 10 days and 11.50 days in BL-4, Thatta-10 and Larkana-2001 respectively with NAA (3 mg l<sup>-1</sup>). Abu *et al.*, (2014) recorded 88 per cent rooting response in NCO-334 variety on media supplemented with 4 mg l<sup>-1</sup> of NAA and 5 mg l<sup>-1</sup> NAA (85.33 per cent) whereas maximum percentage of shoots regenerated roots at 1 mg l<sup>-1</sup> IBA and 1.5 mg l<sup>-1</sup> IBA (85 per cent) for B52-298 variety. According to Tolera (2016) sugarcane variety B41-227 gave maximum ( $33 \pm 0.15$ ) number of roots per shoot, while only  $24.17 \pm 0.00$  roots per shoot were observed in N14 on half strength MS medium with 2 mg l<sup>-1</sup> NAA and 1 mg l<sup>-1</sup> IBA. Sughra *et al.*, (2014) recorded average number of 6.80, 2.5 and 4.90 roots per shoot in BL-4, Thatta-10 and Larkana-2001 respectively with 3 mg-l NAA. Tolera (2016) obtained average root length of  $2.92 \pm 0.18$  cm and  $2.58 \pm 0.00$  cm in B41-227 and N14 respectively. Behera and Sahoo (2009) obtained average root length of  $4.0 \pm 0.94$  cm for the variety Nayana on

MS media supplemented with 2.5 mg l<sup>-1</sup> of NAA. Gopitha *et al.*, (2010) recorded average length of root 4.9 cm in half MS medium supplemented with 3 mg l<sup>-1</sup> of NAA.

Seedlings obtained from leaf roll explants of 87A298 and Co86032 survived better (72.2% and 65.4%) when compared with their counterparts (Table 4). Where as in CoT8201 shoot tip derived explants and in 2003V46 apical meristem derived explants recorded higher survival per cent (56.4% and 64.2%) than the other explants. Whereas, Purushothaman *et al.*, (2000) reported 42 days for acclimatization with high survival percentage on sand, silt and press mud in a ratio of 1:1:1.

Table 1. Callus induction in four sugarcane varieties using three explants

Treatments	Variety/Explant	Mean no. of explants induced callus	Mean no. of days for callus induction	No. of days taken for shoot induction	Callus induction frequency (%)
T <sub>1</sub>	87A298 ST	-	-	36.1	-
T <sub>2</sub>	87A298 LR	5.7	12.8	-	63.00
T <sub>3</sub>	87A298 M	7.4	16.4	-	82.55
T <sub>4</sub>	Co86032 ST	-	-	34.8	-
T <sub>5</sub>	Co86032 LR	6.9	17.2	-	76.66
T <sub>6</sub>	Co86032 M	5.5	15.2	-	61.88
T <sub>7</sub>	CoT8201 ST	-	-	47.7	-
T <sub>8</sub>	CoT8201 LR	4.1	17.2	-	45.67
T <sub>9</sub>	CoT8201 M	5.1	19.5	-	56.25
T <sub>10</sub>	2003V46 ST	-	-	40.3	-
T <sub>11</sub>	2003V46 LR	5.8	17.1	-	63.55
T <sub>12</sub>	2003V46 M	4.6	15.3	-	51.11
	Mean	5.6	16.4	39.7	62.58

ST- Shoot tip, LR- Leaf roll and M- Apical meristem

Table 2. Shoot induction in four sugarcane varieties using three explants

Treatments	Variety/Explant	No. of days for shoot initiation	Shoot regeneration frequency (%)	No. of shoots per explant	Avg. Shoot length (cm)
T <sub>1</sub>	87A298 ST	34.5	86.6 (68.59)	18.4	3.8
T <sub>2</sub>	87A298 LR	28.8	63.3 (53.01)	20.0	3.8
T <sub>3</sub>	87A298 M	31.7	82.2 (65.18)	18.1	3.9
T <sub>4</sub>	Co86032 ST	34.8	67.7 (55.20)	17.2	3.9
T <sub>5</sub>	Co86032 LR	34.6	76.6 (60.89)	18.5	3.9
T <sub>6</sub>	Co86032 M	41.7	61.1 (51.76)	17.2	3.8
T <sub>7</sub>	CoT8201 ST	48.1	61.1 (51.76)	13.6	4.1
T <sub>8</sub>	CoT8201 LR	36.2	45.5 (42.40)	10.8	4.2
T <sub>9</sub>	CoT8201 M	46.1	56.6 (48.77)	9.2	4.3
T <sub>10</sub>	2003V46 ST	38.5	82.2 (65.10)	17.1	3.9
T <sub>11</sub>	2003V46 LR	24.7	64.4 (53.12)	18.6	3.9
T <sub>12</sub>	2003V46 M	27.2	51.1 (45.65)	15.1	3.9
<b>Mean</b>		35.6	66.5	16.2	4.0
<b>C.D at 5%</b>		1.13	1.13	0.99	0.06
<b>SE(m)</b>		0.38	0.38	0.33	0.02
<b>SE(d)</b>		0.54	0.54	0.47	0.03
<b>CV</b>		1.88	1.21	3.61	0.96

Note: Values in parentheses represent arc sine transformed value

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**Table 3. Root induction in four sugarcane varieties using three explants**

Treatments	Variety/ Explant	Time taken for root induction	Root induction frequency	No. of roots produced per shoot	Avg. root length (cm)
T <sub>1</sub>	87A298 ST	8.1	84.91 (67.11)	8.1	1.97
T <sub>2</sub>	87A298 LR	12.1	81.46 (64.47)	9.4	2.48
T <sub>3</sub>	87A298 M	13.1	87.50 (69.30)	8.7	1.96
T <sub>4</sub>	Co86032 ST	11.1	78.01 (62.00)	9.3	1.38
T <sub>5</sub>	Co86032 LR	9.3	77.95 (61.99)	7.8	1.85
T <sub>6</sub>	Co86032 M	12.4	69.32 (56.33)	8.5	1.53
T <sub>7</sub>	CoT8201 ST	14.7	54.17 (47.37)	6.8	2.30
T <sub>8</sub>	CoT8201 LR	10.6	41.08 (39.82)	6.7	3.18
T <sub>9</sub>	CoT8201 M	13.5	47.86 (44.13)	8.4	3.11
T <sub>10</sub>	2003V46 ST	11.2	68.56 (55.87)	7.8	1.77
T <sub>11</sub>	2003V46 LR	14.1	85.06 (67.81)	7.3	1.94
T <sub>12</sub>	2003V46 M	11.0	74.85 (59.87)	9.1	2.10
<b>Mean</b>		11.8	70.89	8.2	2.13
<b>C.D at 5%</b>		0.90	0.61	1.04	0.08
<b>SE(m)</b>		0.30	0.21	0.35	0.02
<b>SE(d)</b>		0.43	0.29	0.50	0.04
<b>CV</b>		4.54	0.62	7.53	2.37

Note: Values in parentheses represent arc sine transformed values

**Table 4. Hardening percentage in four sugarcane varieties using three explants**

Treatments	Variety/Explant	No. of days taken for acclimatization	Survival percentage (%)
T <sub>1</sub>	87A298 ST	35.0	67.0 (54.97)
T <sub>2</sub>	87A298 LR	36.5	72.2 (58.58)
T <sub>3</sub>	87A298 M	36.3	69.1 (56.23)
T <sub>4</sub>	Co86032 ST	41.2	63.8 (53.06)
T <sub>5</sub>	Co86032 LR	42.8	65.4 (54.00)
T <sub>6</sub>	Co86032 M	40.3	61.2 (51.52)
T <sub>7</sub>	CoT8201 ST	44.2	56.4 (48.70)
T <sub>8</sub>	CoT8201 LR	45.7	54.8 (47.78)
T <sub>9</sub>	CoT8201 M	48.3	52.4 (46.38)
T <sub>10</sub>	2003V46 ST	39.0	60.1 (50.83)
T <sub>11</sub>	2003V46 LR	37.7	61.3 (51.54)
T <sub>12</sub>	2003V46 M	38.6	64.2 (53.25)
<b>Mean</b>		40.5	62.3
<b>C.D at 5%</b>		4.07	4.96
<b>SE(m)</b>		1.38	1.68
<b>SE(d)</b>		1.96	2.38
<b>CV</b>		5.93	5.60

Note: Values in parentheses represent arc sine transformed values



## CONCLUSIONS

Differential response for callus induction, shoot induction and root induction was observed among four varieties with three explants. Among all four varieties, 87A298 responded well and CoT8201 did not give better results for callus induction, shoot induction, root induction and hardening. CoT8201 require a standard protocol for in better in vitro response. Hence, by using micropropagation best performing genotypes can be multiplied and commercialized within a short period of time and supplement the conventional propagation which improves both the quality and quantity of the planting materials.

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