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Effect of genotypes on *in vitro* propagation of sugarcane varieties of Andhra Pradesh

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Abstract
Four sugarcane varieties and three explants were utilized to assess better *in vitro* response and observed differential response for callus induction, shoot induction and root induction. *In vitro* performance of 87A298 and 2003V46 was superior over Co86032 and CoT8201. So there is need to have a standard protocol for specific varieties.

Keywords: Sugarcane, *in vitro*, explants

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 method, by the time the varieties start deterioration in yield potential. There are also chances of perpetuation of sett-borne diseases. Micropropagation offers scope for rejuvenating the genetic potential of the varieties by eliminating viruses thereby ensuring supply of healthy seed material and stability in productivity (Sengar *et al.*, 2011) [7].

Materials and Methods
Four popular sugarcane varieties 87A298, Co86032, CoT8201 and 2003V46 were replicated thrice using Completely Randomized Design (CRD) at Agricultural Research Station, Perumallapelle, and Tirupati. 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.
Actively growing points of 8-12 months sugarcane tops were used as explants (shoot tip, leaf roll and apical meristem) and washed them with sterile distilled water thrice then treated with 3 per cent (w/v) sodium hypochlorite ($HgCl_2$) solution for about 10 minutes in laminar air flow chamber and washed out thrice with sterile distilled water.
During present study at different stages of plantlet production certain types of slow growing microbial contaminants persisted even after initial surface sterilization of explants. This was overcome by adding streptomycin to the medium. Browning of culture media was observed near/around the base of the plant due to release of phenols. To avoid this, spindles were subcultured regularly at an interval of 7-10 days by transferring to the fresh medium. PVP and ascorbic acid were added to reduce phenol formation.
For callus, shoot and root induction explants were inoculated on sterilized semisolid basal MS medium supplemented with 2, 4-D (3.0 mg l^{-1}), BAP (3 mg l^{-1}), IAA (2 mg l^{-1}), Kinetin (2 mg l^{-1}) and half strength MS medium supplemented with NAA (2 mg l^{-1}) and sucrose (30 g l^{-1}) respectively. PH of 5.8 was maintained 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 with artificial illumination of 2000-3000 lux by placing the cultures at 25-30 cm below the fluorescent light

and maintained the temperature at 25 ± 2ºC.

Raised seedlings were allowed for hardening with polybags of 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.

Results and Discussion

Mean number of explants that induced callus ranged from 4.1 to 7.4 (Table 1). Maximum number of explants (7.4) that induced callus was recorded in T_3 (87A298 from apical meristem) and T_5 (Co86032 from leaf roll) (6.9). Minimum number (4.1) was recorded in T_8 (CoT8201 from leaf roll) which indicated that CoT8201 required a higher concentration of 2, 4-D for callus induction.

Mean number of days taken for callus induction ranged from 12.8 days to 19.5 days (Table 1). Among all the treatments, maximum number of days (19.5 days) taken for callus induction was recorded in T_9 (CoT8201 from apical meristem).

Callus induction frequency ranged from 45.67 per cent to 82.55 per cent (Table 1). Highest response (82.55 per cent) was recorded in treatment T_3 (87A298 from apical meristem) followed by T_5 (Co86032 from leaf roll) (76.66 per cent). The results revealed that there was slight genotypic difference in callus induction. Similar variation of callus induction response with variety at 3 mg l^{-1} of 2, 4-D was reported by Vu Anh Tuan *et al* (2015) [11].

As shown in (Table 1) significant differences were observed in per cent callus formation among the genotypes. These results revealed the fact that callogenesis response is genotypic dependent. The four genotypes might not be related genetically. It is also varied with the explants. Leaf rolls appeared to be better in Co86032 and 2003V46 and apical meristem in 87A298 and CoT8201. These findings are in agreement with Gandonou *et al.* (2005) [4].

Number of days taken for shoot induction ranged from 34.8 days to 47.7 days (Table 2). Maximum number of days (47.7 days) for shoot induction was recorded in T_7 (CoT8201 from shoot tip). The results are in agreement with that of Goel *et al.* (2015) [6] and Garcia *et al.* (2007) [5] for shoot induction number of days taken for shoot initiation ranged from 24.7 days to 48.1 days (Table 2). Minimum number of days (24.7 days) for shoot initiation was recorded in T_{11} (2003V46 from leaf roll).

Shoot induction frequency ranged from 45.5 per cent to 86.6 per cent. (Table 2). The maximum shoot induction frequency (86.6 per cent) was recorded in T_1 (87A298 from shoot tip). Among all treatments of 87A298, T_1 (87A298 from shoot tip) recorded maximum shoot induction frequency (86.6 per cent). Among all treatments of Co86032, T_5 (Co86032 from leaf roll) recorded maximum shoot induction frequency (76.6 per cent). Among all treatments of CoT8201, T_7 (CoT8201 from shoot tip) recorded maximum shoot induction frequency (61.1 per cent). Among all treatments of 2003V46, T_{10} (2003V46 from shoot tip) recorded maximum shoot induction frequency (82.2 per cent). The results were in accordance with Tripathi and Lal (2013) [10].

This results indicated that shoot tip explants is far better for culture establishment. The established culture showed vigorous and bunchy shoots. Leaf roll and meristem culture were taken more time for establishment in match up to shoot tip explants. Higher responses regarding the frequency of shoot initiation and establishment in shoot tip than in meristem explants suggested that large size of explant have endogenous growth regulators (cytokinins) and nutrients, which help in survival of explant while meristem explant is comparatively smaller in size.

The number of shoots produced per explant was ranged from 9.2 to 20.0 (Table 2). Maximum number of shoots per explant (20.0) was recorded in T_2 (87A298 from leaf roll). Among four varieties, mean number of shoots per explant was maximum in 87A298. Among all the treatments of 87A298, T_2 (87A298 from leaf roll) recorded maximum number of shoots per explant (20.0). Among all the treatments of Co86032, T_5 (Co86032 from leaf roll) recorded maximum number of shoots per explant (18.5). Among all the treatments of CoT8201, T_7 (CoT8201 from shoot tip) recorded maximum number of shoots per explant (13.6). Among all treatments of 2003V46, T_{11} (2003V46 from shoot tip) recorded maximum number of shoots per explant (18.6). Yadav *et al.* (2012) [12] reported similar results of shoot regeneration.

Maximum average shoot length (4.3 cm) was recorded in T_9 (CoT8201 from meristem) (Table 2). Among four varieties, CoT8201 recorded maximum shoot length even though lower shoot regeneration frequency and number of shoots per explant. Similar findings were in agreement with Sughra *et al.* (2014) [8].

The time taken for root induction ranged from 8.1 days to 14.7 days. (Table 3). Maximum number of days (14.7 days) was recorded in T_7 (CoT8201 from shoot tip). Out of four varieties, minimum mean number of days for root induction was recorded in Co86032. Within 87A298 variety, T_1 (87A298 from shoot tip) recorded minimum number of days (8.1 days) for root induction than other explants. Within Co86032 variety, T_5 (Co86032 from leaf roll) recorded minimum days (9.3 days) for root induction. Within CoT8201 variety, T_8 (CoT8201 from leaf roll) recorded minimum days (10.6 days) for root induction. Among all the treatments of 2003V46, T_{12} (2003V46 from apical meristem) recorded minimum days for root induction. These results were supported by Sughra *et al.* (2014) [8] for root induction.

Root induction frequency ranged from 41.08 per cent to 87.50 per cent (Table 3). Maximum root induction frequency (87.50 per cent) was recorded in T_3 (87A298 from apical meristem). In all four varieties, 87A298 noticed maximum (87.50 per cent) mean rooting frequency in T_3 (87A298 from apical meristem). Among all the treatments of Co86032, T_4 (Co86032 from shoot tip) recorded high rooting frequency (78.01 per cent). Among all the treatments of CoT8201, T_7 (CoT8201 from shoot tip) recorded high rooting frequency (54.17 per cent) and among all the treatments of 2003V46, T_{11} (2003V46 from leaf roll) recorded high rooting frequency (85.06 per cent). Root induction frequency in all the explants of 87A298 was good and with respect to CoT8201 it was poor.

Hence, it is obvious from the above result that percentage of shoots regenerating root is genotype specific with respect to different growth regulators. The above results can be complemented by Yadav *et al.* (2012) [12] and Abu *et al.* (2014) [1].

Number of roots produced per shoot was ranged from 6.7 to 9.4 (Table 3). Maximum number of roots per shoot (9.4) was recorded in T_2 (87A298 from leaf roll). Among four varieties, mean number of roots per shoot was observed to be maximum in 87A298. Among all the treatments of 87A298, T_2 (87A298 from leaf roll) recorded maximum number of roots per shoot (9.4). Among all the treatments of Co86032, T_4 (Co86032 from shoot tip) recorded maximum number of roots per shoot (9.3). Among all the treatments of CoT8201, T_9 (CoT8201

from apical meristem) recorded maximum number of roots per shoot (8.4) and among all the treatments of 2003V46, T_{12} (2003V46 from apical meristem) recorded the maximum number of roots per shoot (9.1). Similar findings were reported by Sughra *et al.* (2014) [8] for this character

Average root length ranged from 1.38 cm to 3.18 cm (Table 3). Maximum average root length (3.18 cm) was recorded in T_8 (CoT8201 from leaf roll). Among all the four varieties, mean average root length was maximum in CoT8201. Among all the treatments of 87A298, T_2 (87A298 from leaf roll) recorded maximum average root length (2.48 cm) than other treatments of 87A298. Among all the treatments of Co86032, T_5 (Co86032 from leaf roll) recorded maximum average root length (1.85 cm) than other treatments of Co86032. Among all the treatments of CoT8201, T_8 (CoT8201 from leaf roll) recorded maximum average root length (3.18 cm) than the other treatments of CoT8201. Among all the treatments of 2003V46, T_{12} (2003V46 from apical meristem) recorded maximum average root length (2.10 cm) than other treatments of 2003V46. The results were in agreement with the findings of Tolera (2016) [9] for root length.

The effect of variations in the concentrations and combination of the same hormone in most of the cited literatures and in the present work is almost entirely due to variation in the varieties of sugarcane tested by different researchers. That is why it is of paramount importance to optimize genotype specific *in vitro* propagation protocols for every variety.

The number of days taken for hardening ranged from 35 days to 48.3 days (Table 4). Minimum number of days (35 days) for hardening was recorded in T_1 (87A298 from shoot tip). Among four varieties, mean number of days for hardening was minimum in 87A298. Among all the treatments of Co86032, T_6 (Co86032 from apical meristem) recorded minimum number of days (40.3 days) for hardening. Among all the treatments of CoT8201, T_7 (CoT8201 from shoot tip) recorded minimum number of days (44.2 days) for hardening. Among all the treatments of 2003V46, T_{11} (2003V46 from leaf roll) recorded minimum number of days (37.7 days) for hardening. Similar results were reported by Ali *et al.* (2008) [2] for number of days for hardening

Hardening per cent ranged from 52.4 per cent to 72.2 per cent (Table 4). The maximum hardening per cent (72.2 per cent) was recorded in T_2 (87A298 from leaf roll). Among four varieties, mean hardening per cent was as maximum in 87A298. Among all the treatments of Co86032, T_5 (Co86032 from leaf roll) recorded maximum (65.4 per cent) hardening per cent. Among all the treatments of CoT8201, T_7 (CoT8201 from shoot tip) recorded maximum (56.4 per cent) hardening per cent. Among all the treatments of 2003V46, T_{12} (2003V46 from apical meristem) recorded maximum (64.2 per cent) hardening per cent. Dinesh *et al.* (2015) [3] reported same results for this character.

The reason for lower acclimatization response may be associated with the environment in which the varieties were acclimatized. During the experiment, factors such as humidity, temperature, light intensity, soil type and other factors affecting acclimatization were not precisely measured or considered well. Besides, there may be varietal difference for acclimatization response as compared to other varieties tested in the previous studies.

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_1	87A298 ST	-	-	36.1	-
T_2	87A298 LR	5.7	12.8	-	63.00
T_3	87A298 M	7.4	16.4	-	82.55
T_4	Co86032 ST	-	-	34.8	-
T_5	Co86032 LR	6.9	17.2	-	76.66
T_6	Co86032 M	5.5	15.2	-	61.88
T_7	CoT8201 ST	-	-	47.7	-
T_8	CoT8201 LR	4.1	17.2	-	45.67
T_9	CoT8201 M	5.1	19.5	-	56.25
T_{10}	2003V46 ST	-	-	40.3	-
T_{11}	2003V46 LR	5.8	17.1	-	63.55
T_{12}	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_1	87A298 ST	34.5	86.6 (68.59)	18.4	3.8
T_2	87A298 LR	28.8	63.3 (53.01)	20.0	3.8
T_3	87A298 M	31.7	82.2 (65.18)	18.1	3.9
T_4	Co86032 ST	34.8	67.7 (55.20)	17.2	3.9
T_5	Co86032 LR	34.6	76.6 (60.89)	18.5	3.9
T_6	Co86032 M	41.7	61.1 (51.76)	17.2	3.8
T_7	CoT8201 ST	48.1	61.1 (51.76)	13.6	4.1
T_8	CoT8201 LR	36.2	45.5 (42.40)	10.8	4.2
T_9	CoT8201 M	46.1	56.6 (48.77)	9.2	4.3
T_{10}	2003V46 ST	38.5	82.2 (65.10)	17.1	3.9
T_{11}	2003V46 LR	24.7	64.4 (53.12)	18.6	3.9
T_{12}	2003V46 M	27.2	51.1 (45.65)	15.1	3.9
Mean		35.6	66.5	16.2	4.0
C.D at 5%		1.13	1.13	0.99	0.06

SE(m)		0.38	0.38	0.33	0.02
SE(d)		0.54	0.54	0.47	0.03
CV		1.88	1.21	3.61	0.96

Note: Values in parentheses represent arc sine transformed value

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_1	87A298 ST	8.1	84.91 (67.11)	8.1	1.97
T_2	87A298 LR	12.1	81.46 (64.47)	9.4	2.48
T_3	87A298 M	13.1	87.50 (69.30)	8.7	1.96
T_4	Co86032 ST	11.1	78.01 (62.00)	9.3	1.38
T_5	Co86032 LR	9.3	77.95 (61.99)	7.8	1.85
T_6	Co86032 M	12.4	69.32 (56.33)	8.5	1.53
T_7	CoT8201 ST	14.7	54.17 (47.37)	6.8	2.30
T_8	CoT8201 LR	10.6	41.08 (39.82)	6.7	3.18
T_9	CoT8201 M	13.5	47.86 (44.13)	8.4	3.11
T_{10}	2003V46 ST	11.2	68.56 (55.87)	7.8	1.77
T_{11}	2003V46 LR	14.1	85.06 (67.81)	7.3	1.94
T_{12}	2003V46 M	11.0	74.85 (59.87)	9.1	2.10
Mean		11.8	70.89	8.2	2.13
C.D at 5%		0.90	0.61	1.04	0.08
SE(m)		0.30	0.21	0.35	0.02
SE(d)		0.43	0.29	0.50	0.04
CV		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_1	87A298 ST	35.0	67.0 (54.97)
T_2	87A298 LR	36.5	72.2 (58.58)
T_3	87A298 M	36.3	69.1 (56.23)
T_4	Co86032 ST	41.2	63.8 (53.06)
T_5	Co86032 LR	42.8	65.4 (54.00)
T_6	Co86032 M	40.3	61.2 (51.52)
T_7	CoT8201 ST	44.2	56.4 (48.70)
T_8	CoT8201 LR	45.7	54.8 (47.78)
T_9	CoT8201 M	48.3	52.4 (46.38)
T_{10}	2003V46 ST	39.0	60.1 (50.83)
T_{11}	2003V46 LR	37.7	61.3 (51.54)
T_{12}	2003V46 M	38.6	64.2 (53.25)
Mean		40.5	62.3
C.D at 5%		4.07	4.96
SE(m)		1.38	1.68
SE(d)		1.96	2.38
CV		5.93	5.60

Note: Values in parentheses represent arc sine transformed values

Conclusions

Four popular sugarcane varieties of Andhra Pradesh comprised of genetic potential though showed reduced performance due to pathogen accumulation. In this context plant regeneration was undertaken using explants (shoot tip, leaf roll and apical meristem). Out of four varieties, 87A298 responded well and CoT8201 does not given better results. There by standard protocol is required for better *in vitro* response for CoT8201. Hence, by using micro propagation best performing genotypes can be multiplied and commercialized within a short period of time and supplement the conventional propagation ultimately improves quality and quantity of the planting materials.

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