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RESEARCH PAPER

Effect of media concentration and growth hormones on shoot regeneration and *in vitro* rooting of sugarcane varieties (*Saccharum* spp.)

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Abstract : The protocol for plantlet induction and regeneration through *in vitro* culture was standardized for the sugarcane variety Co 06022 with Co 86032. Genotype specific media combinations for establishment and rooting efficiency was identified and standardized. Multiple shoot regeneration was observed at different frequencies, using different concentration and growth regulator combination. *In vitro* propagation of *Saccharum* offers opportunities for increasing plant material for cultivation. Apical meristems were cultured on modified MS medium containing different concentrations of auxins. In Co 06022, the most effective concentration was combination of ½ MS medium supplemented with IBA (2.0 mg/l) which induced an average of 3.00±0.14 number of shoots per microshoot with 10.77±0.42 cm shoot length. Among the auxins used, NAA showed better response than IBA for profuse rooting in Co 06022. The number of roots formed was maximum (14.80±0.09) in MS with NAA (2mg/l) which had taken 10 days for root initiation with the root length of 6.45±0.07 cm. However, in Co 86032, the best performance was observed on ½ MS medium supplemented with IBA (2.0 mg/l) with more number of shoots (2.00 ± 0.19) and average shoot length of 12.30± 0.44 cm. Best rooting was observed in ½ MS medium supplemented with NAA (2.0 mg/l) and maximum number of roots were 11.60±0.12 which has taken 8 days for initiation of root primordia with average root length of 1.16±0.12 cm. Thus, it can be deduced that this protocol can be used successfully for *in vitro* rooting and acclimatization of these genotypes.

Key Words : Sugarcane, Micropropagation, Shoot induction, Root induction

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INTRODUCTION

Globally, sugarcane (*Saccharum* spp.) is a major sugar and cash crop, accounting for around 70 per cent of the total production of sugar in the world (FAO, 2019). Most sugarcane farmers face problems with low cane yields due to poor quality seed materials, disease and pest prevalence and slow commercialization of improved sugarcane varieties. Usage of an effective propagation system is, therefore, a must for mass multiplication of disease-free seed material to mitigate these problems.

Currently, micro propagation is a powerful tool for the continuous supply of newly improved varieties which

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plays a major role in the rapid multiplication of disease-free planting material (Nasir *et al.,* 2014). Micro propagation is a relatively easy *in vitro* process for the rapid propagation of clean or pathogen-free plant materials and conservation of germplasm of selected sugarcane materials (Nickell and Heinz, 1973; Lorenzo *et al.,* 2001; Raman and Lal, 2004 and Biradar *et al.*, 2009).

In accordance with this, protocols have been developed for rapid multiplication of newly released and commercially important genotypes of sugarcane through micro propagation method. The ultimate success of commercial micro propagation depends largely on successful rooting and acclimatization of *in vitro* derived plantlets. Rooting process is genetically determined and influenced by culture environment factors such as mineral concentration, type and concentration of growth regulators and sucrose concentration in the culture media. Thus genotype specific protocol is needed as the plant growth regulators and sucrose requirements for morphogenetic responses of *in vitro* rooting vary from genotype to genotype in sugarcane (Sood *et al*., 2006 and Tesfa *et al*., 2016).

Auxins are a class of phytohormones used for rooting in *in vitro* cultures. Indole -3 –butyric acid (IBA) is often the desirable auxin for rooting purposes and was found to be the most favourable root inducer compared to Idole-3-Acetic Acid (IAA) and Naphthalene Acetic acid (NAA) (Yasodha *et al.,* 2008). IAA is a natural auxin but it is unstable (Bonga and Aderkas, 1992) and IBA is a more easily metabolized auxin with a slow release effect, whereas NAA, a highly stable auxin, remains in the tissues in free form and blocks root emergence (Fogaca and Neto, 2005). The selection of a suitable combination is one of a decisive factor which substantially affects the survival rate, growth and development of *ex vitro* acclimatized plantlets and mainly depends on plant species. Therefore, the study was carried out with objectives to optimize *in vitro* rooting protocol for two elite sugarcane genotypes Co 86032 and Co 06022.

MATERIAL AND METHODS

Plant material and explant source:

Sugarcane varieties, Co 86032 and Co 06022 were obtained from breeder seed plot maintained in Sugarcane Breeding Institute, Coimbatore. These plants served as the source for explants in *in vitro* rooting studies. Shoot tips were collected from 5-6 months old plants with 5 cm in size.

Inoculation medium:

Surface sterilized shoot tips were inoculated in the basal medium with salts and vitamins supplemented with different concentrations of plant growth regulators, sucrose and agar. The pH was adjusted to 5.8 prior to autoclaving at 121°C for 15 minutes at 15 p.s.i. After 14 days of inoculation in the M1 and M2 medium, the shoots were transferred to the shoot regeneration medium.

Shoot regeneration:

The basal medium (liquid) was supplemented with different concentrations and combinations of IBA (0, 0.25, and 0.5 mg L^{-1}) for shoot regeneration. The cultures were incubated at 25±2°C under dark condition for two weeks, followed by 16/8 h photoperiod (light/dark) and the light intensity of 40 µmol m $2s^{-1}$ provided by cool white fluorescent lamps during shoot regeneration. Explants were sub cultured at four weeks intervals and data were recorded after 10 and 14 weeks of culture.

Root induction:

After sub culturing, the regenerated *in vitro* shoots from regeneration experiment were cultured into the liquid MS medium (full strength and half strength) with different concentrations (NAA and IBA 1.0 and 2.0 mg/l) for *in vitro* rooting. Cultures were transferred onto new medium with the same constituents in four-week intervals for two times. All rooting cultures were kept under the 16/8h photoperiod (light/dark) and the light intensity of 2000-3000 lux provided by white fluorescent lamps. Details of the treatments were given in the Table A.

Table A: Rooting media of regenerated shoots for Co 86032 and Co 06022 with different combinations of MS media and growth hormones

Sr. No.	Treatment code	Composition	Conc. (mg/L) of hormones
1.	A	½ MS+NAA	1.0
2.	B	½ MS+NAA	2.0
3.	C	MS+NAA	1.0
4.	D	MS+NAA	2.0
5.	E	½ MS+IBA	1.0
6.	F	½ MS+IBA	2.0
7.	G	MS+IBA	1.0
8.	H	MS+IBA	2.0
9.	Control	MS+IAA+IBA	2.0+1.0+1.0

Statistical analysis

Completely Randomized Design (CBD) was set up in an experiments and each experiment had 10 replicates and was repeated 2 times. In each replication, 10 explants were used per treatment. Observation was recorded in terms of shoot length (SL-cm), root length (RL-cm), number of shoots/ micro shoot (NS), number of roots (NR), root angle (RA) and days to root formation (DRI) in both the sugarcane varieties. Duncan's Multiple Range Test (DMRT) was used to compare means at 5 per cent probability level according to Gomez and Gomez (1976).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Effects of NAA and IBA on shoot regeneration:

Shoot tips incubated on MS medium supplemented with various concentrations of NAA (1–2mg/L) and IBA (1-2mg/L) were able to regenerate shoots of varieties Co 86032 and Co 06022.The meristematic shoot tips elongated over two weeks and axillary buds emerged from the leaf axils after four weeks of inoculation. Axillary shoots increased in height and adventitious shoots began to regenerate. Initially, shoot induction was very slow, but after 28 days new shoots were regenerated and it is grew on low concentration of growth regulators.

The results of analysis of variance on shoot regeneration indicated that the number of shoots (NS), number of roots formed (NR), root angle (RA), root length (RL) and days of root initiation (DRI) were significantly influenced by the application of NAA and IBA in both the varieties of sugarcane (Co 86032 and Co 06022). All combinations of NAA and IBA were able to regenerate shoots from shoot tip explants either with full strength or half strength MS media (Table 1).

Among different concentrations and combinations for shoot multiplication, best performance was observed on ½ MS medium (F) supplemented with IBA (2.0 mg/l) (Table 2). On this combination, the number of shoots

Table 1 : Analysis of variance for six characters of Co 86032 and Co 06022

Traits	Co 86032			Co 06022		
	Treatment	Replication	Error	Treatment	Replication	Error
df	8	26	18	8	26	18
SL	9.01	5.54	3.99	31.84**	9.75	0.09
RL	0.43**	0.15	0.03	6.29**	1.92	0
NR	15.87**	4.91	0.03	36.74**	11.2	0.03
RA	352.39**	108.45	0.032	266.99**	81.27	0.01
NS	0.69**	0.24	0.05	1.04**	0.34	0.04
DRI	8.83**	2.73	0.01	11.75**	3.58	0.01

** indicate significance of value at P=0.05

Table 2: Effect of different concentration and combination of IAA and IBA on shoot initiation of sugarcane varieties

Treatments	Hormone	Hormonal supplement (mg/L)	Co 86032		Co 06022	
			Shoot length (cm)	No. of shoots/ Micro shoot	Shoot length (cm)	No. of shoots/ Micro shoot
A	1/2 MS+NAA	1.00±0.15^{b}	11.93±0.06^{a}	1.20±0.02^{bc}	16.33±0.27^{a}	2.17±0.06^{cb}
B	1/2 MS+NAA	2.00±0.21^{a}	10.47±5.95^{c}	1.22±0.03^{b}	9.20±0.37^{f}	2.50±0.11^{b}
C	MS+NAA	1.00±0.25^{b}	12.00±0.07^{a}	1.00±0.31^{bc}	9.60±0.23^{f}	1.50±0.11^{d}
D	MS+NAA	2.00±0.25^{a}	11.73±0.32^{a}	1.00±0.21^{bc}	6.37±0.32^{g}	2.86±0.24^{ab}
E	1/2 MS+IBA	1.00±0.35^{b}	9.53±0.09^{d}	1.80±0.08^{a}	15.30±0.42^{b}	1.67±0.20^{d}
F	1/2 MS+IBA	2.00±0.26^{a}	12.30±0.44^{a}	2.00±0.19^{a}	10.77±0.42^{e}	3.00±0.14^{a}
G	MS+IBA	1.00±0.40^{b}	9.67±0.34^{d}	0.87±0.22^{c}	11.87±0.07^{c}	1.50±0.36^{d}
H	MS+IBA	2.00±0.21^{a}	11.21±0.37^{b}	2.00±0.11^{a}	11.21±0.13^{d}	1.75±0.18^{d}
Control	MA+IAA+IBA	2.0+1.0+1.0	10.02±0.08	1.00±0.42	10.00±0.04	1.80±0.20
C.D. (p<0.05)		0.4711	0.4361	0.368	0.5218	0.3378

Values are mean ± SD of three values Mean values with in a column no common superscript differ significantly at 5% by DMRT

was 2.00 ± 0.190 with average shoot length of 12.30± 0.44 cm in Co 86032. The second best performance was found on MS medium (H) supplemented with IBA (2.0mg/l) in which the number of shoots/ microshoot was 2.00±0.11 with the shoot length 11.21±0.08cm. In Co 06022, the most effective concentration was combination of ½ MS medium (F) supplemented with IBA (2.0 mg/l) which induced an average of 3.00±0.14 number of shoots per microshoot with 10.77±0.42 cm shoot length. The second best concentration for number of shoots was MS medium (D) supplemented with 2mg/L of NAA (2.86±0.24). The previous findings of Islam *et al.* (1982) reported the positive effects of BAP+IBA combination on shoot formation in sugarcane. The best response in terms of multiple shoot induction was observed on MS medium supplemented with BAP 2.0mg/L+IBA 0.5mg/ L. Among various concentrations of BAP used 1.0, 1.5 and 2.0mg/l in growth medium supported efficient regeneration and plenty of lateral shoots in a minimum time span almost in all cultivars of sugarcane (Jahangir *et al.,* 2014). Previous studies on different cultivars of sugarcane concluded that regeneration potential of callus was specific and genotype dependent phenomenon and at the same time it is in parallel with the hormonal concentration and combinations (Maretzki and Nickell, 1973; Maretzki, 1987; Behera and Sahoo, 2009 and Dinesh *et al.,* 2015).

In vitro rooting:

For rooting studies, the regenerated microshoots were further multiplied on liquid MS medium with different types of auxins at different concentrations and combinations to induce adventitious roots in micro propagated shoots. In Co 86032, best rooting was observed in ½ MS medium (B) supplemented with NAA (2.0 mg/l) and maximum number of roots was found to be 11.60±0.12 which has taken 8 days for initiation of root primordia with average root length of 1.16±0.12 cm. The second best performance was observed in MS medium (H) supplemented with IBA (2.0 mg/l). It has recorded 10.80±0.25 number of roots with 2.07±0.07cm root length with 9 days for root primordia initiation. The per cent of establishment was > 50% in both the treatments.

Among the auxins used, NAA showed better response than IBA for profuse rooting in Co 06022. The number of roots formed was maximum (14.80±0.09) in MS (D) with NAA (2mg/l) which had taken 10 days for root initiation with the root length of 6.45±0.07 cm. The second best treatment for more number of roots (11.47±0.14) was MS medium (C) supplemented with NAA (1.00mg/l). The root length was 6.45± 0.07cm with 10 days of root initiation (Table 3). Present results confirm the findings of Behera and Sahoo (2009) who reported 2.5mg/l NAA to be more effective supplement for higher number of adventitious root formation in sugarcane cultivar Nayana. When *in vitro* shoot lets were inoculated on to the half-strength semi solid MS medium supplemented with 2.5mg/l NAA, rooting was more profuse (Dinesh *et al*., 2015 and Tesfa *et al.,* 2016). These findings also agree well with the previous findings of Nadar and Heinz (1977). Baksha *et al*. (2002) found

Table 3 : Effect of different concentration and combination of IAA and IBA on root initiation of sugarcane varieties

Description				Co86032			Co 06022			
Treatments	Hormone	Hormonal supplement (mg/L)	Root length (cm)	No.of roots formed	Days to root initiation	% root induction	Root length (cm)	No.of roots formed	Days to root initiation	% root induction
A	1/2 MS+NAA	1.00±0.15^{b}	1.33±0.09^{c}	6.20±0.12^{f}	7.00±0.15^{e}	46.60±17.16	4.30±0.06^{d}	10.20±0.14^{c}	7.00±0.16^{e}	46.65±0.14^{e}
B	1/2 MS+NAA	2.00±0.21^{a}	1.16±0.12^{c}	11.60±0.12^{a}	8.00±0.08^{d}	53.30±0.18^{c}	5.80±0.13^{b}	10.20±0.43^{c}	8.00±0.05^{d}	53.48±0.20^{d}
C	MS+NAA	1.00±0.25^{b}	1.15±0.15^{c}	8.13±0.11^{d}	6.00±0.08^{f}	40.00±0.06^{e}	5.23±0.08^{c}	11.47±0.14^{b}	6.00±0.07^{e}	40.15±0.07^{f}
D	MS+NAA	2.00±0.25^{a}	0.93±0.06^{c}	9.13±0.12^{c}	7.00±0.12^{e}	46.60±0.13^{d}	6.45±0.07^{a}	14.80±0.09^{a}	10.00±0.09^{c}	66.60±0.15^{c}
E	1/2 MS+IBA	1.00±0.35^{b}	1.73±0.38^{b}	7.00±0.08^{e}	8.00±0.15^{d}	53.30±0.14^{c}	4.03±0.06^{e}	4.80±0.09^{f}	10.00±0.05^{c}	66.60±0.09^{c}
F	1/2 MS+IBA	2.00±0.26^{a}	1.56±0.23^{b}	9.13±0.16^{c}	9.00±0.09^{b}	60.00±0.06^{b}	3.83±0.08^{f}	7.40±0.12^{d}	6.00±0.10^{e}	40.27±0.16^{f}
G	MS+IBA	1.00±0.40^{b}	1.33±0.17^{c}	4.20±0.28^{g}	8.00±0.11^{d}	53.45±0.25^{c}	3.70±0.10^{f}	6.47±0.04^{e}	7.00±0.15^{e}	46.68±0.14^{e}
H	MS+IBA	2.00±0.21^{a}	2.07±0.07^{a}	10.80±0.25^{b}	9.00±0.12^{b}	60.04±0.13^{b}	1.73±0.03^{g}	5.00±0.14^{f}	11.00±0.07^{b}	73.35±0.19^{b}
C	MA+IAA+IBA	2.0+1.0+1.0^{a}	0.87±0.17^{c}	7.00±0.20^{c}	12.00±0.05^{a}	80.00±5.24^{a}	0.75±0.02	8.50±0.18^{d}	13.00± 0.12^{a}	86.60± 0.26^{a}
S.E.±		0.2222	0.150	0.140	0.089	0.1504	0.0637	0.1519	0.0814	0.1208
C.D. (p<0.05)		0.4711	0.315	0.295	0.187	0.3160	0.1350	0.3220	0.1725	0.2561

Values are mean ± SD of three values Mean values with in a column no common superscript differ significantly at 5% by DMRT

that 5.0 mg/l NAA for best rooting response in half strength MS medium for sugarcane. Jagadeesh *et al.* (2011) reported the rooting can best be achieved in half strength MS medium supplemented with 6.0 mg /l NAA alone. Jahangir *et al*. (2014) found that for rhizogenesis, 5.0mg/l of IAA was found to be most efficient among four different concentrations of auxin. Some cultivars of sugarcane have a sufficient endoauxin level and do not need any supplementation for rooting *i.e.,* basal medium supports root induction.

Conclusion:

Our findings showed that in micro propagation the shoot elongation and root induction in sugarcane are influenced by genotype and culture media. Growth regulator concentrations used for micro propagation also differently affects the root induction in the sugarcane varieties. The genotypes used in this study reacted well to micro propagation protocol in terms of shoot and root induction. Maximum root induction and root length was observed in the composition, MS medium with NAA (2mg/l) for the new variety Co 06022 when compared to Co 86032. Among the auxins used, NAA showed better response than IBA for profuse rooting in Co 06022. Further, the media compositions were equally important and, out of the media tested in this study, the best media for *in vitro* rooting and shoot elongation ½ MS medium supplemented with IBA (2.0 mg/l). The study will fill the knowledge gap and will be helpful in deciding the suitable media composition for rapid multiplication technique that enables to minimize the time and cost required for large-scale propagation of newly evolved and high yielding sugarcane genotypes. The study gives an idea on genotype specific regeneration and rooting efficiency under different media combinations.

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