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ffect of media composition on *in vitro* multiplication of sugarcane ırieties

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Abstract: The *in vitro* response of sugarcane varieties CO 86249 and CO 8021 was assessed on twelve media for callus induction, multiplication, shooting and rooting. CO 86249 recorded the highest callusing frequency at 2,4-D 3 mg/lit. with 0.1 mg/lit casein hydrolysate followed by 2,4-D 3 mg/lit with 100 ml of coconut milk. CO 86249 had significant shoot multiplication ratio and length in MS media supplemented with1 mg/lit IBA+ 2.0 mg/lit+1.0 mg/lit kinetin. The half strength MS media supplemented with 1 mg/lit NAA+ 1.25 mg/lit IBA was promoted profuse root in both the varieties. Whereas, full strength MS media along with different levels of growth hormone showed reduction in number of roots in both the varieties.

Key words : Saccharum officinarum, callusing, shooting, rooting in vitro.

troduction

Sugarcane is the main yielding crop grown tropical and subtropical areas of the world. dia is the largest producer of sugar in the ɔrld (Shahi,1999) because sugarcane is the ıly source to meet its sugar demand, which ɔccupies 4,049 mha of land area. Sugar industry s the second largest agro-industry in India, vhich shares two per cent of the Gross Domestic Product (GDP). The projected sugarcane production ɔy the year 2020 is estimated as 415 mt. With the limited area we have to produce the argeted yield in sugarcane. This can be achieved ɔy evolving elite genotypes with high yielding ɔotential. As sugarcane is clonally propagated :rop only limited genetic variation could be ɔbtained. So, induction of variations through he application tissue culture is a potential tool for the crop improvement. Keeping this in view, the present investigation was carried out in plant tissue culture laboratory, department of Plant breeding and Genetics, Agricultural College and Research Institute, Madurai during 2000-2001 to fix the media composition for the development of sugarcane variety.

Materials and Methods

Young healthy immature spindle leaves (0.5 - 1.00 cm length) of two sugarcane varieties CO 86249 and CO 8021 were used as explant for induction of callus and shoot. Stock solutions were prepared in double distilled water and kept in refrigerator at 5°C for further use. The nutrient medium used for this study was MS medium, which contained inorganic salts and sucrose as carbon source. The hormone used was 2,4-D at different concentration (2.0, 2.5, 3.0, 3.5 mg/lit) in addition with some organic additives *viz.*, casein hydrolysate (0.1 g/lit) and coconut milk (100 ml/lit). Twelve different combinations were tried to fix the suitable media combination for callus induction. Healthy and disease free spindle leaves are separated out carefully without any damage. The spindle leaves were subjected to series of sterilization *viz.*, first the leaf bits were placed in 70 per cent alcohol for 30 seconds, then the leaf bits were taken out and exposed for complete evaporation of alcohol, transferred to sterile water to remove the excess alcohol adhering on it, followed by shaking in the petridish containing 0.1 per cent mercuric chloride for surface sterilization and finally the leaf bits were washed two to three times in sterile distilled water for complete removal of mercuric chloride. After sterilization the leaf bits were inoculated in test tubes having twelve different media composition, keeping twenty tubes in each combination per replication. Three replications were maintained. After inoculation, tubes were kept inn dark at pa temperatuer of 24±1°C for about 2-4 weeks.

Table 1. Percentage of callus induction

Treatments	Varieties	
	Co 86249	Co 8021
2, 4-D		
2.0 mg/lit	40.46	36.91
2.5 mg/lit	45.00	40.21
3.0 mg/lit	54.85	43.40
3.5 mg/lit	48.20	41.81
2, 4-D+CH		
2.0 mg/lit + 0.1 g/lit	49.85	45.00
2.5 mg/lit + 0.1 g/lit	63.94**	45.00
3.0 mg/lit + 0.1 g/lit	61.97**	54.85
3.5 mg/lit + 0.1 g/lit	60.22*	60.22*
2, 4-D+CM		
2.0 mg/lit + 100 ml/lit	46.60	43.40
2.5 mg/lit + 100 ml/lit	54.85	56.49
3.0 mg/lit + 100 ml/lit	66.38**	55.00
3.5 mg/lit + 100 ml/lit	60.22*	48.25
* Significant at 5% level	** Significant at 1% level	
SEd	3.658	
CD (0.05)	7.357	
CD (0.01)	9.814	

Table 2. Effect of media on shoot multiplication and length of shoot in sugarcane

No.	Growth regulator (mg/lit)			No.of shoots		Shoot length (cm)	
	IBA	BA	Kinetin	Co 86240	Co 8021	Co 86240	Co 8021
1	0.5	1.0	0.5	1.45	1.58	2.89	3.21
2	0.5	2.0	0.5	2.39	1.63	3.32	3.04
3	0.5	1.0	1.0	1.70	1.56	3.92	4.08
4	0.5	2.0	1.0	3.59**	3.38	4.81**	4.26*
5	1.0	1.0	0.5	2.90	1.93	4.03	3.31
6	1.0	2.0	0.5	.43**	2.60	4.91**	4.18
7	1.0	2.0	1.0	4.89**	4.09**	5.28**	5.02**
8	1.0	2.0	1.0	5.30**	4.48**	6.70**	5.78**
9	Control	1.17	1.09	1.80	2.02		
SEd		0.11		0.10			
CD (0.05)		0.24		0.22			
CD (0.01)		0.33		0.30			

* Significant at 5% level ** Significant at 1% level

The callus derived from various media compositions were used for shooting. The dried calli were removed and the fresh calli of uniform size were taken and transferred to MS medium supplemented with growth regulator *viz.* two levels of kinetin (0.5 and 1.0 mg/lit), IBA (0.5 and 1.0 mg/lit) and BA (1.0 and 2.0 mg/lit), thus made eight treatment combinations and a control without growth regulator. Studies were carried out to know the effect of different media on shoot multiplication and length of shoots and frequency of shoot multiplication.

Table 3. Effect of growth hormones on root induction in half and full strength of MS media

Hormones combination	Concentration of hormones (mg/lit)	No. of roots after 3 weeks			
		Half MS media		Full MS media	
		Co 86249	Co 8021	Co 86249	Co 8021
NAA + IBA	0.5 + 0.5	5.85	4.41	3.42	3.19
	0.5 + 1.0	9.22	8.12	6.78	5.12
	0.5 + 1.25	10.81**	11.17**	8.41**	7.12**
	0.5 + 1.5	10.41**	9.16	7.07**	6.47
NAA + IBA	1.0 + 0.5	8.61	6.42	5.72	4.40
	1.0 + 1.0	12.11**	9.34	7.48**	5.88
	1.0 + 1.25	14.11**	12.02**	9.36**	7.73**
	1.0 + 1.5	12.7**	10.39**	8.10**	7.12**

* Significant at 5% level ** Significant at 1% level

SEd	0.413	0.222
CD (0.05)	0.876	0.471
CD (0.01)	1.207	0.649

Twenty tubes per replication in each media composition for each variety with two replication were taken.

After subculturing the test tube were incubated in a culture room at 26±1°C and were exposed to 2000 lux light intensity for 16 h per day. Regeneration was observed carefully. After 35 days, observations were made on number of shoots and length of shoot.

Later plantlets were selected and transferred into half strength MS media without kinetin and supplemented with 0.5 mg/lit and 1.0 mg/lit NAA along with different concentrations (0.5, 1.0, 1.25 and 1.5 mg/lit) of IBA, thus made eight different rooting media composition. Full strength MS media with all the eight combination were also separately studied. After three weeks, observations were recorded on number of roots produced.

Results and Discussion

Callus Induction

In most of the cases with increase in 2,4-D concentration percentage of callus induction also increases up to certain level (2.0-3.0 mg/lit). In the case of 2,4-D alone without additives both varieties responded well up to 3.0 mg/lit (Table 1). Ceullar, (1997) reported addition of 2,4-D up to 3.0 mg/lit for the development of calli from young rolled leaves of sugarcane. Addition of casein hydrolysate (0.1 g/lit), the variety CO 86249 recorded high percentage of callus induction (63.94%) at 2,4-D 3.0 mg/lit, while CO 8021 (60.22%) with 2,4-D 3.5 mg/lit (Table 1).

Among the sugarcane varieties Co 86249 showed good response and recorded high percentage of callus induction (66.38) at 2,4-D 3.0 mg/lit in addition with 10 per cent (100 ml/lit) coconut milk (Prajapati *et al.* 2001). Whereas CO 8021 recorded good induction percentage (56.49) at 2.5 mg/lit of 2,4-D with coconut milk (100 ml/lit) (Table 1). Jimenez Gonzalez *et al.* (1990) observed that the coconut milk had a similar effect as that of auxin.

Multiple shoots obtained after 35 days

Different levels of IBA, BA and kinetin had effective influence on shoot multiplication irrespective of the variety. The variety, treatment and variety x treatment interaction were significant. The highest significant shoot multiplication ratio was observed in treatment combination of 1.0 mg/lit IBA+2.0 mg/lit BA + 1.0 mg/lit kinetin followed by 1.0 mg/lit IBA+1.0 mg/lit BA + 1.0 mg/lit kinetin combination (Table 2).

Among the varieties, CO 86249 recorded highly significant shoot multiplication ration (1:5.3). The lowest shoot multiplication ratio was observed in the variety CO 8021 (1:1.09) and CO 86249 (1:1.17) at control. Whereas, the treatment combination 0.5 mg/lit IBA + 1.0 mg/lit BA + 0.5 mg/lit kinetin was observed to be less effective. This was in accordance with the results obtained by Patel *et al.* (2001) and Prasad and Chaturvedi (1992).

Length of shoot

Both the varieties of sugarcane CO 86249 (6.7 cm) and CO 8021 (5.78 cm) produced lengthy shoot in a treatment combination of 1.0 mg/lit IBA + 2.0 mg/lit BA+ 1.0 mg/lit kinetin followed by 5.28 cm in CO 86249 and 5.02 cm in CO 8021 on 1.0 mg/lit IBA+1.0 mg/lit BA+1.0 mg/lit kinetin. The lowest length of shoot was developed by the control as 1.80 cm (CO 86249) and 2.02 cm (CO 8021) (Table 2). The similar findings were recorded by Prasad and Chaturvedi (1992) in their experiment.

Root multiplication

Among the varieties, CO 86249 produce profuse rooting at half strength MS media with a hormone level of 1 g/lit NAA + 1.25 mg/lit IBA (14.11) followed by 1 mg/lit NAA + 1.5 mg/lit IBA (10.87). Reduction in number of roots was observed with increased level of IBA beyond 1.25 mg/lit. In the case of CO 8021 more number of roots were produced at the above said level as 12.02 and 11.17 respectively. Full strength MS media along with growth hormone at all the levels showed reduction in root numbers campare to half strength media Prajapati *et al.* (2001) observed similar results in their studies (Table 3).

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