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Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane

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ABSTRACT

Studies on micropropogation of callus culture was undertaken. In which expalnts were inoculated in MS medium fortified with various concentration of 2,4-D, auxin, cytokinin, sucrose at different pH level .The best callus induction was observed at 3.0mg/L, 2,4-D with 10% coconut milk (CM). Best regeneration of shoot was achieved when they were cultured on MS medium supplemented with BAP 1.0mg/L and IBA 0.5mg/L. Among the different media tested with 3mg/L NAA and 5% sucrose supplemented media proved best production of roots.

KEY WORDS

MS medium –Murashige and skoog medium BAP - 6-Benzyl amino purine; Kn - kinetin: IBA- Indole-3-Butyric acid: NAA -Napthalene acetic acid: CM - coconut milk

INTRODUCTION

Sugar cane (*Saccharum officinarum*) is one of the most important cash crops in India majorly used to produce cheap food in the form of sugar and gur that further lends itself for energy reproduction. In order to meet the demands it is urgent to increase cane productivity without the expansion of area. Plant regeneration through tissue culture technique would be a better alternative for improving the quality and production. Micropropogation method is an effective method for rapid propogation of sugarcane . The initial attempts were made by Nickell (1964) and Heinz & Mee (1969).

Tissue culture of sugarcane has received considerable research attention because of its economic importance as a cash crop. During the last two decades the technique of plant tissue culture has developed as a new and powerful tool for crop improvement (Carlson, 1975). Under proper condition the differentiation of either shoots or roots from callus of most sugarcane varieties is easily obtained (Heinz and Mee, 1969) Leaf sheath were used as explants to induce callus on modified MS supplemented with 2,4-D as growth regulators (Karim *et al*, 2002. In this present study various concentration of auxins (IBA & NAA) and cytokinins (BAP & Kn) are used to study the induction of callus and shoot regeneration in sugarcane.

MATERIALS AND METHOD

Explant for callus formation: Very young expanding scaling leaves of sugarcane cultivar (CO 671) of 3-5 months old plants were selected. The outer whorls are removed and apical part of shoots were trimmed and cut into 12cm length. Then surface sterilized with 0.1% HgCl2 for seven minutes followed by four washings with sterile distilled water under aseptic condition. The outer layers were removed and the innermost five leaf whorls were cut into 1cm^2 bit and they were inoculated in MS (Murashige & Skoog,1962) media fortified with different concentration of 2,4,D; IBA & NAA (0.5,1.0, 2.0,3.0,4.0, 5.0)

Shoot regeneration from the callus tissue of sugarcane: The callus induced tissues were transferred to MS media supplemented with different concentration of cytokinin (BAP and Kn) [0.5,1.0,2.0 mg/l] and different combination of BAA + NAA [0.5 + 0.1 – 2.0 + 1.0 mg/L] and BAP + IBA [0.5 + 0.1 – 2.0 +1.0 mg/L].

Rooting of invitro grown microshoot culture : The invitro grown microshoot were inoculated into the ½ strength MS media supplemented with different concentration of Auxin IBA,NAA,IAA [0.5,1.0,3.0,5.0,7.0] and a combination of NAA + IBA [0.5 + 0.5 ,0.5+1.0,1.0 + 0.5,1.0 + 1.0,3.0+0.5,3.0+1.0,5.0+0.5,5.0+0.5,5.0+1.0,7.0+0.5,7.0+1.0]. Simultaneously the effect of sucrose concentration along with the different pH range on rooting medium was studied by supplementing different concentration of sucrose [10,15,20,25,30,35,40,45,50,55,,60,65,70] and adjusting the medium at different pH levels [3.0,3.5,4.0,4.5,5.0,5.5,6.0,6.5,7.0 mg/L].

RESULT

Callus formation: Callus formation was observed in 15 days after inoculation from the leaf sheath explants on modified MS medium containing different concentration of IBA, NAA and 2,4- D [0.5,1.0,2.0,3.0,4.0,5.0 mg/L]. Maximum callus induction was observed at 3.0mg/L 2,4-D with 10% coconut milk [Table-1], minimum callus induction was observed at 1.0 mg/L of NAA. And no callus induction was seen in any of the concentrations of IBA.

Shoot regeneration from callus: Among the different concentration and combination of BAP, Kn, BAP +NAA and BAP+IBA for shoot, the highest shoot regenerating capacity (94%) was recorded in 1.0+0.5 mg/L (BAP+ NAA) combination. The minimum (32%) shoot regeneration was observed in 1.0+1.0 mg/L (BAP+NAA) combination. In BAP+IBA combination 1.0+0.5mg/L combinations showed maximum (95%) of root produced, whereas the minimum was noticed in 2.0+1.0mg/L combinations. In case of BAP 1.0mg/L showed maximum (74%) of shoot production. Whereas the minimum (18%) was noticed in 0.5mg/L concentration and with Kn the media showed maximum (40%) of shoot production and the minimum (12%) was noticed in 0.5-mg/L concentration. Among the all-experimental combination 1.0+0.5 (BAP+NAA) and 1.0+0.5mg/L (BAP+IBA) showed better performance in sugarcane shoot regeneration. The average number of shoots in BAP + NAA (1.0 + 0.5mg/L) was 15.5 and BAP + IBA (1.0 + 0.5mg/L) was 6.4 respectively (Table 2).

Rooting of invtro microshoot culture/ Root formation: Among different concentration and combination of Auxin, NAA and IBA were found to be comparatively better response than IAA for producing roots.NAA + IBA combination showed positive result. Best rooting (95%) was observed in ½ strength of MS medium supplemented with 3mg/L NAA [Table-3] and the highest number of average roots per micro shoots and average length of root were 15 and 4.9 cm. The plantlets with well developed roots were successfully transplanted in soil and the percentage survivability was 70. In case of IBA the best rooting (88%) was observed in concentration 3mg/L and highest number of average roots/microshoots were 12.6 and the average length of roots were 3.7cms. In combination of NAA+IBA best rooting (85%) was observed at concentration 3.0 + 1.0 mg/L and the highest number of average roots/microshoots were 14.5 and the average length of roots was 4.1. The poor response of minimum or no development was noticed in media supplement with 0.5mg/L , 7.0mg/L of IAA and NAA + IBA with concentration of 0.5+0.5mg/L and 7.0+1.0mg/L.

Different concentration of sucrose: Sugar cane microshoots originated from callus inducted tissue could be rooted on media containing a wide range [0,10,15,20,25,30,35,40,45,50,55,60,65,70g/L] of sucrose. The maximum percentage (96%) of microshoots rooted were seen in the media supplement with sucrose concentration of 50gms/L. However the rooting percentage on media containing no sucrose or very high level concentration (70g/L) of sucrose were lower, and showing 20% of microshoots rooted and 42% microshoots rooted in them respectively (Table - 4).

Different pH level: The pH of the medium may be limiting factor for growth media, pH was adjusted between 3.0 and 7.0. Among these pH level the highest percentage (96%) rooting was recorded on the medium adjusted to pH 5.7 and plants showed poor

quality of rooting at pH 3.5,4.0,6.5 and 7.0 respectively, no roots were formed at pH 3.0 (Table-5).

DISCUSSION

Sugar cane (*Saccharum officinarum*) is a major agricultural crop in the tropical and subtropical regions of the world. Sugarcane varieties are highly heterogenous and generally multiplies vegetatively by stem cutting. Lack of suitable multiplication procedure has long been serious problem in sugarcane breeding programme (Nand & Singh,1994)

Plant tissue culture offers the best methodology through micropropogation of sugarcane for quality and phytosanitary planting material at a faster rate in a shorter period of time.

Callus induction was observed within two weeks time after inoculation from the leaf sheath explants on modified MS medium containing different concentration of IBA,NAA and 2,4 _D (0.5 – 5.0 mg/L).Though callus induction was triggered in all; best callus induction was observed at 3.0mg/L 2,4-D with 10% cm, on this media composition the explant produced greenish white color callus. The percentage of callus induction was 85% maximum at 3.0mg/L 2,4-D with 10% cm. Begum etal, (1995) found 3-5mg/L of 2,4-D produced highest percentage of callus induction in Bangladeshi sugarcane varieties (Viz Nagarbari, L.jaba,isd – 16,isd-20 and clone I.123).

Kambaska kumar Behra *et al* .(2009) standardized a protocol for induction of callus and regeneration of plantlets through invitro culture using young meristem of sugarcane *(Saccharum officinarum* L.*cv - Narayan*) as an explants. The multiple shoot regeneration at various frequencies was observed by using different concentration and combination of growth regulators. The highest percentage of callus induction was observed in MS supplemented with 2.5mg/L ; 2,4-D.The best response in terms of multiple shoot induction was observed on MS with BAP 0.2mg/L + NAA 0.5mg/L.

Rapid micropropogation of three elite sugarcane varieties (HSF –240, CP – 77-400 and CPF – 237) by shoot tip culture was carried out using liquid MS medium containing 2% sucrose supplemented with BAP in combination of GA3. And optimum multiplication was observed at 1mg/L BAP in combination with 0.1 mg/L GA3 for variety HSF – 240, for CP 77 – 400- 0.5mg/l BAP + 0.1mg/L GA3 and for CPF – 237 – 1.0mg/L BAP + 0.5mg/L GA3.Rootin was observed on ½ strength liquid MS medium with 6% sucrose containing different concentration of IBA and NAA (Sabaz Alikham *et al* , 2008)

Induction of callus regeneration response of two sugar cane varieties (isd-16, isd-28) was established through callus culture using leaf sheath.The highest percentage of callus induction was observed in medium containing 3.0mg/L 2,4-D with 10%cm.The best response in terms of multiple shoot formation was observed on MS medium supplemented with BAP 1.0mg/L + IBA 0.5mg/L; NAA (3.0mg/L) was found to be effective in production of roots. The variety isd –16 showed better response than variety isd -28 towards shoot multiplication (M.Z.Karim *et al,* 2002).

The study of micropropogation has given a rapid technology compared with conventional technique for multiplication and germplasm preservative of elite sugarcane varieties. Therefore callus induction and regeneration of shoots and roots offers a definite scope for further improvement of this adapted genotype through gene manipulation using other biotechnological techniques.

Table-1
Effect of different concentration, auxin and 2,4-D on callus induction from leaf sheath explants of sugarcane

Name of the Hormone	Concentration Mg/L	No.of explants inoculated	No.of explants showed callus	% of explants with callus induction
IBA	0.5	20	-	-
	1.0	20	-	-
	2.0	20	-	-
	3.0	20	-	-
	4.0	20	-	-
	5.0	20	-	-
	0.5	20	-	-
NAA	1.0	20	-	-
	2.0	20	1	5
	3.0	20	2	10
	4.0	20	-	-
	5.0	20	-	-
2,4-D	0.5	20	3	15
	1.0	20	9	45
	2.0	20	15	75
	3.0	20	17	85
	4.0	20	8	40
	5.0	20	-	-

"-" = No callusing;
Poor callusing =20-50%;
Considerable callusing =51-85%;
Intensive callusing =86-100%.

Table-2

Effect of the cytokinin (BAP,Kn) and the auxin (IBA,NAA) at different concentrations and combination is Ms medium on shoot on shoot regeneration from the callus tissue of sugarcane

Name of the hormone	Concentration Mg/L	% of explants produced shoot	Average no.of shoot per explants	Average no of the usual shoot cm
BAP	0.5	18	3.5	3.8
	1.0	74	6.6	3.3
	2.0	51	4.5	3.0
Kn	0.5	12	2.5	3.0
	1.0	40	4.3	2.9
	2.0	32	2.2	3.5
	0.5+0.1	48	3.5	3.1
	0.5+0.2	62	6.4	3.3
	0.5+0.5	35	9.7	2.0
	0.5+1.0	20	10.3	2.6
	1.0+0.1	60	11.4	2.8

BAP + NAA	1.0+0.2	72	11.6	4.6
	1.0+0.5	94	15.5	5.3
	1.0+1.0	32	5.4	2.4
	2.0+0.1	45	4.6	2.9
	2.0+0.2	66	3.7	4.3
	2.0+0.5	50	2.8	3.1
BAP + IBA	2.0+0.1	42	2.8	2.0
	0.5+0.1	52	2.5	4.0
	0.5+0.2	59	4.5	3.2
	0.5+0.5	65	5.2	4.1
	0.5+1.0	71	7.1	4.5
	1.0+0.1	75	6.0	5.7
	1.0+0.2	84	5.5	3.3
	1.0+0.5	95	6.4	3.8
	1.0+1.0	49	3.3	2.0
	2.0+0.1	40	3.4	2.2
	2.0+0.2	33	2.6	2.1
	2.0+0.5	31	2.5	2.3
	2.0+1.0	29	1.5	2.0

BAP = 6-Benzyl amino purine
Kn = kinetin;
IBA = Indole-3-Butyric acid;
NAA = Napthalene acetic acid

TABLE – 3

Effect of different auxin on formation of root on the invitro grown micro shoots Cultured

Name of the Hormone	Concentration Mg/L	% of micro shoots Rooted	Average No.of roots/micro shoots	Average length of roots- cm
IBA	0.5	33	8.2	1.5
	1.0	65	10.5	2.4
	3.0	88	12.6	3.7
	5.0	73	8.4	3.5
	7.0	45	6.3	2.1
NAA	0.5	42	10.4	1.0
	1.0	63	11.6	1.4
	3.0	95	15.1	4.9
	5.0	80	9.6	3.9
	7.0	54	8.8	3.8
IAA	0.5	-	-	-
	1.0	20	5.5	1.3
	3.0	55	7.4	1.6
	5.0	30	4.5	2.2
	7.0	-	-	-
	0.5+0.5	-	-	-
	0.5+1.0	45	6.3	2.2
	1.0+0.5	52	7.2	2.6
	1.0+1.0	63	9.1	3.9

NAA + IBA	3.0+0.5	80	12.3	3.5
	3.0+1.0	85	14.5	4.1
	5.0+0.5	54	6.8	2.0
	5.0+1.0	65	6.3	2.2
	7.0+0.5	50	5.5	1.4
	7.0+1.0	-	-	-

Table-4

Effect of sucrose concentration on rooting of invitro differentiated shoots of sugarcane

S.No	Sucrose concentration g/L	% of micro shoots rooted	Average no.of roots per shoots	Average length of roots-cm
1.	Nil	20	1.2	1.5
2.	10	32	3.6	1.7
3.	15	40	4.1	2.5
4.	20	48	5.5	3.4
5.	25	62	7.2	3.9
6.	30	76	8.4	4.2
7.	35	79	11.1	4.1
8.	40	85	12.8	4.4
9.	45	91	15.9	4.6
10.	50	96	17.3	4.8
11.	55	89	15.3	4.2
12.	60	74	11.5	3.8
13.	65	62	8.5	3.2
14.	70	42	3.8	2.0

Table -5

Effect of different pH level on rooting of invitro differentiated Shoots of sugarcane

S.No	pH level	% of micro shoots rooted	Average no. of root per shoots	Average length of roots-cm.
1.	3.0	-	-	-
2.	3.5	20	0.9	0.8
3.	4.0	32	1.2	1.6
4.	4.5	48	2.7	1.8
5.	5.0	76	7.9	1.9
6.	5.5	85	15.1	3.8
7.	5.7	96	17.01	4.6
8.	6.0	78	10.2	2.5
9.	6.5	46	7.3	1.5
10.	7.0	20	2.3	0.6

Sucrose and NAA used were 50g/L and 5.0mg/L respectively.

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