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EFFECT OF HORMONES FOR THE DEVELOPMENT OF *SACCHARUM OFFICINARUM* (SUGARCANE) EMPLOYING TISSUE CULTURE TECHNOLOGY

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Abstract: Plant tissue culture research is multi-dimensional and it has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry. The crop sustains second largest organized agro-industry, the sugar industry. The media combination used for initial establishment phase is MS Basal + 0.5 mg/lit BAP + 0.5 mg/lit Kn + 30 gm/lit Sugar + 8.0 gm/lit agar. And the rooting response found in media combinations having MS + 5 mg/lt NAA + 0.5 mg/lt sugar and as compared to the media with same conc of Auxin but reduced sugar(30gm/lit). After the inoculation of the nodal segments it was found that the sterilization protocol followed for the sterilization of the explants was reliable, as out of 12 explants, only two had reported the contamination so the survival rate comes out to be 83.33%.

Keywords: *Saccharum officinarum, In-vitro,* Rooting, and Shooting.

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INTRODUCTION

The technique of plant tissue culture may play a key role in the "Second Green Revolution'' in which biotechnology and gene modification are being used to improve crop yield and quality. Tissue culture also found wide application in the fields of forestry and biodiversity conservation. Mass production of plantlets for afforestation programme depends greatly in advancement of tissue culture techniques. Plant tissue culture research is multi-dimensional and it has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry. The techniques include culture of cells, anthers, ovules and embryos on experimental to industrial scales, protoplast isolation and fusion, cell selection and meristem and bud culture. Instead of being a tool for rapid propagation, tissue culture became an alternating tool of breeding by selecting and testing for useful variants (Heinz *et al* 1971, Babra *et al* 1977, Morales *et al* 1989). Regeneration of the plants from callus is found to be an important source of variation (Skirvin and janick, 1976). This phenomena is called as some clonal variation which can also be used for the creation of new plant varieties with increased importance (Evans and Sharp 1986). Plant tissue culture has also been used in the production of flavours, sweeteneners, natural colourants and Bio-pharmacuticals (Khann.P 1989)

In our country, agriculture is not an agri-business, but way of life . Sugarcane, an agro-industrial crop, is an important integral component of the agriculture. It assumes the important position in the economy by contributing nearly 1.9% of National GDP. Sugarcane is cultivated in over 4 million hectares spread over a wide range of agro-ecological situations, both in tropical and sub-tropical regions. The crop sustains second largest organized agro-industry, the sugar industry. This has enabled us to be the largest producer of sugar and the second largest producer of sugarcane in the world.

Red rot, caused by Glomerella tucumanensis, (previously known as Colletotrichum falcatum), is one of the major constraints in the profitable cultivation of sugarcane. This fungal disease drastically retards the yield and considerably deteriorates the juice quality (Agnihotri, 1990) thus causing considerable loss for both the growers and millers. Many wonder varieties (e.g. CoJ 64) have now gone out of cultivation due to serious infestation of red rot. The symptoms of the disease first appear when the crop is about six months old.At the initial stage, drying of top leaves can be seen. The leaf startswithering and drooping almost all shoots in a clump starts drying one by one. Red lesions with straw coloured centres develop on the midrib of leaves.

Plant regeneration from tissue culture of sugarcane has been successfully applied to breeding programs for rapid screening of clones for disease resistance, salt tolerance, drought tolerance, herbicide resistance and early maturity and high sugar.

MATERIAL AND METHODS

Collection of Sample

We collected the plant material from Jawaharlal Nehru Agricultural University Jabalpur (M.P.) from the Department of Plant Breeding. The explants from 6-8 month old, healthy, disease free plant canes of variety var CoJ 85 were collected.

Medium

The basal medium employed for the culture of sugarcane is MS medium (Murashige and Skoog 1962). A variety of growth regulators such as 6-Benzyl amino purine (BAP), alpha-Naphthalene acetic acid (NAA), 3-Indole Butyric acid (IBA) and 2,4dichlorophenoxy acetic acid (2,4-D) were added to the medium singly or in combinations at various concentrations and were used for initiating different experiments. The concentrated stock solutions of the major salts, minor salts and vitamins are prepared to be used in the preparation of the media and stored under refrigeration. Auxins were dissolved in 1N KOH and cytokinins in 1N HCL before making up the final volume with distilled water. Iron EDTA stock solution was stored in amber coloured bottle.

Choice of Ex-plant

The tissue taken from a plant and transferred to a culture medium to establish a tissue cultures system or regenerates a plant. The choice of the explant depends upon the methods of the shoot multiplication to be followed. All plant organs viz. nodal segments, internodal segments, shoot tip, root, cotyledons, epicotyl, hypocotyl, leaf, etc are known to give rise to complete plants. For micro propagation, where aim is to get identical plants, it is advisable to initiate cultures from explant from preexisting meristems. It is necessary to know the origin (variety, cultivar). In adult plant to such explants exists i.e. shoot tip and nodal explant (stem portion to which leaf is attached). For most micro propagation work the explant of choice is an apical or an axillary bud. Usually the explant are more responsive to culture treatments if they are collected during the period of active growth.

Washing of the ex-plant

Isolated nodal segments were cleaned under running tap water for about 15 to 20 min. each under laminar air flow hood and followed by three times rinsing in sterile distilled water. Nodal segments were further soaked in fungicide (Bavistin) solution (.5 mg/L) for 10 min. and then again washed with sterile distilled water. Finally .1 g/L mercuric chloride solution for 5 min. was used to treat these explants followed by three times washes with sterile distilled water for complete sterilization of nodal explants. Completely sterilized explants were inoculated on establishment Patil *et al.* 18131 media.

RESULTS AND DISCUSSION

INITIATION

Effect of cytokinins on direct shoot initiation from Explants of Sugarcane

The shoot initiation started within 5-6 days of inoculation. Results obtained indicate that low (0.2mg/lit.), moderate (0.3mg/lit) as well as high (0.5mg/lit) concentrations of BAP are suitable for shoot initiation from apical bud. Similar results were obtained with KN whose low (0.2mg/lit), moderate (0.3mg/lit) as well as high (0.5mg/lit) concentrations proved equally suitable for bringing about shoot initiation. The explants remained fresh and initiated shoot formation within 3-4 days of their inoculation.

Apical bud cultured on MS medium supplemented with KN (0.3mg/lit) together with BAP (0.2-0.3mg/lit) resulted in a low percentage of shoot emergences within 7-8 days of culture. In all the explants heavy swelling of the hypocotyl accompanied shoot initiation. A decreasing trend of shoot initiation was recorded (75% -50%) with the increase in concentration of BAP in this combination.

Table No. 1 Effect of cytokinins (alone and in combination) on direct shoot initiation from Explants of Sugarcane

S.No.	PGR Concentration (mg)	% Shoot initiation (SI)
		Ap B
	Control	
1.	0.0	45.2
	BAP	
2.	0.2	75
3.	0.3	85
4.	0.5	100
	KN	
5.	0.2	75
6.	0.3	85
7.	0.5	100
	KN+BAP	
8.	0.2+0.2	65

9.	0.2+0.3	70
10.	0.2+0.5	70

ELONGATION

Effect of cytokinins on shoot Elongation from Explants of Sugarcane

Cytokinins were applied to elongate the shoots initiated from the apical buds. Elongation was observed in most of the PGR concentrations. Apical buds cultured on basal medium showed shoot elongation in only 57.14% cases with average shoot length 'reaching 2.61 cm. Shoot elongation was characterized by increase in number of nodes in the regenerated shoots. All concentrations of BAP and KN showed a decrease in shoot elongation with the increase in concentrations of cytokinins. The low concentrations of BAP (0.2mg/lit.) and KN (0.2mg/lit.) alone as well as moderate concentrations of both BAP (0.2mg/lit) and KN (0.2mg/lit) turned out to be more suitable for shoot elongation. Whereas the higher concentrations of both BAP (0.5mg/lit) and KN (0.5mg/lit) were not much suitable. A decrease in the average length of shoots ranged from 8.0cm-5.9cm on BAP (0.2-0.5mg/lit) and 8.9 cm-5.25cm on KN (0.2-0.5mg/lit) supplemented media respectively. In BAP supplemented media, the shoot elongation was good and the shoots were thick. Leaf was healthy, broad and moderate sized as compared to that on KN. All the shoots base where the hypocotyl made a contact with the medium showed swelling tendency within 5-6 days followed by rapid callusing of the basal portion. The basal callus was of regenerative type; when these callus was scraped out during sub culturing and was inoculated on basal medium or on low concentrations of BAP it gave rise to shoot like structures. Some of elongated shoots from apical buds on BAP and KN supplemented media, showed drying or necrosis. The axillary buds in these cases got initiated and give rise to shoots and roots No elongation was observed in the treatment involving combinations of the cytokines (KN +BAP).

Table 2 Effect of cytokinins (alone and in combination) on shoot elongation from Explants of Sugarcane

S.No.	PGR Concentration (mg)	% Shoot Elongation (SE)	MSL (cms)
		Ap B	
	Control		
1.	0.0	45.2	2.61
	BAP		

2.	0.2	75	5.0
3.	0.3	85	6.2
4.	0.5	100	9.0
	KN		
5.	0.2	75	5.0
6.	0.3	85	5.1
7.	0.5	100	8.2
	KN+BAP		
8.	0.2+0.2	65	5.7
9.	0.2+0.3	70	5.9
10.	0.2+0.5	70	8.1

After 30 days of incubation the sprouted bud had grown considerably especially in the medium containing MS + 0.5 mg/lit BAP + 0.5 mg/lit Kn. The length and basal growth of the sprouted buds are observed significantly higher than the other medium containing MS + 1.0mg/lit BAP+ 1.0 mg/lit Kn. bly especially in the medium containing MS + 0.5 mg/lit BAP + 0.5 mg/lit Kn. The length and basal growth of the sprouted buds are observed significantly higher than the other medium containing MS + 1.0mg/lit BAP+ 1.0 mg/lit Kn.

ROOTING

In case of rooting experiments, the best results were obtained in the medium that was supplemented with MS + NAA (5mg/l) and which had elevated levels of sugar (70gm/l). It was found that roots initiate after 8-10 days and sufficient rooting was obtained within 14-15 days after inoculation into the medium. These newly formed roots showed good growth and were slightly white to pinkish in colour and normal in appearance. The roots were approximately 3.8 cm in length and rooting percentage is approximately 85% for the same medium. It was important to note that most of the shoots that were inoculated into the medium showed considerable root formation within 15 days. Some of references cited here have also indicated that the use of NAA is desirable for the best response to rooting in case of sugarcane. (Singh, B. Yadav, G. C. Lal, M., Sugar Tech, 2001).

Table 3. Observation of Rooting of shoots

MEDIUM	No. of rooted shoots (14 days)	Av. root length (14 days)
A) 5 NAA (Full strength MS) + 70gm/lit Sugar	13.33 ± 0.6	3.86 ± 0.1
B) 3 NAA+ 2 IBA (Full strength MS) + 70 gm/lit Sugar	11.67 ± 0.6	3.73 ± 0.06
C) 0.5 IBA (Half strength MS) + 30 gm/lit Sugar	10.67 ± 0.6	2.86 ± 0.1

TRANSPLANTATION TO THE POLYBAGS

After the sufficient rooting of the sugarcane cultures they were then transferred to the polybags that were having peat and then watered adequately. Due to the lack of time we were not able to find out the proper

Hardening of the sugarcane plantlets:

After 15 days of culture of microshoots on rooting medium, which resulted in the sufficient rooting of shoots, the plantlets were transplanted to plastic pots containing garden soil and Farmyard manure (1:1) for their hardening. For first ten- days the plantlets were kept in polyhouse. To maintain the appropriate humidity level (80%), plants were thoroughly watered with the help of manual 36 sprinkler every 2 hours The temperature of polyhouse was maintained at 31^{o}C with humidity level of nearly 80%.

Table 4. Survival rate of plantlets of *Sugarcane* at different stages of Hardening.

Stage of transplantation	Number of plants Transplanted	Percentage of Survival
Poly House (1st stage)	13	85
Shade House* (2nd stage)	11	82

*Plantlets transferred to shade house after 10 days growth on Polyhouse

CONCLUSION

In this present study, we have attempted to work on Tissue culture protocol for Sugarcane, which is an important crop for Indian farmers. Though there are several problems associated with Sugarcane in relation with cultivation, identification of suitable variety in different agro geographical regions, sale of Sugarcane to the mills, minimum support price for the farmers, and a declining trend of Sugarcane cultivation in Indian

We observe and conclude the following in our present study:

1. In identifying the sterilization protocol for sugarcane explant(var CoJ 85), our experience with the followed protocol is satisfactory .

2. We have also observed that the explants have to be subcultured for 3-4 times (in 7-10 days interval) for at least 2 weeks in solid medium before transfer to the solid medium for further establishment. The media combination used for initial establishment phase is MS Basal + 0.5 mg/lit BAP + 0.5 mg/lit Kn + 30 gm/lit Sugar + 8.0 gm/lit agar.

3. Significant rooting response found in media combinations having MS + 5 mg/lt NAA + 0.5 mg/lt sugar and as compared to the media with same conc of Auxin but reduced sugar(30gm/lt) has a reduced response for rooting.

4. We have transplanted a few plants for hardening but due to time constraints we could complete the experiments. To standardize a successful Tissue culture protocol, successful hardening of microshoots are very essential and further experiments are needed.

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