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RESEARCH PAPER

Cheaper carbon sources for micropropagation of banana cv. 'GRANDE NAINE'

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Abstract : Micropropagation of banana has become a routine procedure but high production cost is limiting the commercial use of tissue culture technology. Analytical grade sucrose is the most commonly used carbon source for the micropropagation banana, however, the cost is too high to justify the use at commercial scale. Therefore, inexpensive and readily available sources of carbon such as laboratory grade sucrose, common grade sugar, cube sugar, rock sugar, candy sugar, glucose, jaggery and sugarcane juice were evaluated for *in vitro* propagation of banana cv. 'GRANDE NAINE'. Best response in terms of shoot multiplication and rooting were achieved with rock sugar and common grade sugar, respectively which could be compared well with that of analytical grade sucrose. The results showed the possibility of successful use of cheaper carbon sources for micropropagation of banana cv. 'GRANDE NAINE'.

Key Words : Analytical grade sucrose, Common grade sugar, Grande Naine, Micropropagation, Rock sugar

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INTRODUCTION

Banana is a dessert fruit for millions and is also used in different regions as a staple food owing to its rich and easily digestible carbohydrates. It is propagated conventionally through suckers because being triploid plant, seed setting and propagation by seed is not possible. The major problem in propagation through conventional method is the transmission of soil borne disease through rhizome and viral infection causing bunchy top. Besides, this method is slow and season bound. *In vitro* propagated plants are increasingly becoming the planting material of choice because of disease control, uniformity and rapid multiplication. However, growers have to face higher costs and pay upto five times more than for suckers. Sucker derived banana is still in demand owing to low cost and easy availability. The cost of fully hardened banana is Rs. 12-18/plant while the cost of sucker derived banana is Rs. 4-5/plant. Today only big farmers can afford the micropropagated plants. The high cost of plant is largely due to high price of tissue culture grade sucrose, gelrite and artificial light (Kodym and Zapata-Arias, 2001).

Sucrose has been reported to be the best carbon and energy source (George, 1993). Although sucrose is the commonly used carbohydrate in the vast majority of

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work on *in vitro* shoot induction and development in woody species, it is not always the most effective carbon source for these purposes (Thompson and Thorpe, 1987). Thus the carbohydrate requirements must be defined and optimized for each micropropagation system (Debnath, 2005). In spite of the wide-spread use, the cost of refined sucrose is far too high to justify the use at commercial scale. Sucrose accounts for 21.70 per cent the media cost (Prakash, 1993). The cost of banana tissue culture was successfully reduced by 90 per cent by replacing the tissue culture grade sucrose with a commercial sugar (Zapata, 2001).

The present study was, therefore, undertaken with a leading commercial variety 'Grande Naine' with the objective to identify inexpensive alternative carbohydrates substrates.

MATERIAL AND METHODS

The present study was conducted at the Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences, GKVK, Bangalore, India. Healthy and vigorously growing sword suckers of cv. 'GRANDE NAINE' (3-4 month age), free from viruses and other diseases were selected as a source of explant (Fig. A i).

Preparation of explant :

The plant material obtained from the field was thoroughly washed in running tap water followed by washing with a detergent solution to remove adhering soil particles. Later, rhizomes were kept immersed in a fungicide solution of 1 per cent bavistin for half an hour, to further clean the planting material. The outer leaves, leaf base and corm tissue were trimmed using a sterilized stainless steel knife until the length of explant was 4-6 cm and the diameter, 3-4 cm. These trimmed suckers enclosing the shoot tip were washed with double distilled water. After trimming one more outer layer, they were soaked in a solution of 0.50 % bavistin + 0.05 % streptocycline for eight hours. After thoroughly washing with double distilled water, they were trimmed again, so that trimmed suckers were of 2-3 cm in length and 2-2.5 cm in diameter. These shoot tips were soaked in 0.05 per cent cetrimide for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with 0.1 per cent mercuric chloride in a closed container for 10 minutes. Further operations such as washing several times with sterile distilled water to remove all traces of chlorine, trimming of explants and inoculation were carried out under laminar air flow chamber.

Initiation of aseptic culture :

Shoot tip explants were incubated in MS liquid medium containing 2 mg/lit BAP and 35 mg/lit adenine sulphate for two weeks maintaining standard culture conditions of 25 ± 2^0 C temperature, 70 per cent RH and photoperiodic cycle of 16 hours light and 8 hours dark period (Fig. A ii-iii).

After two weeks of incubation, all the explants (Fig. A iv) were evaluated for their ability to establish in liquid medium. Greening and swelling of the explants were utilized as important criteria for assessing the success in establishment. Shoot tips that had turned dark brown/ black and which did not swell were considered as non-established. Healthy and contaminant free explants were excised by removing discoloured tissue and transferred to the semisolid medium supplemented with BAP (2 mg/ lit) and adenine sulphate (35 mg/lit) and incubated for four weeks maintaining standard culture conditions. The

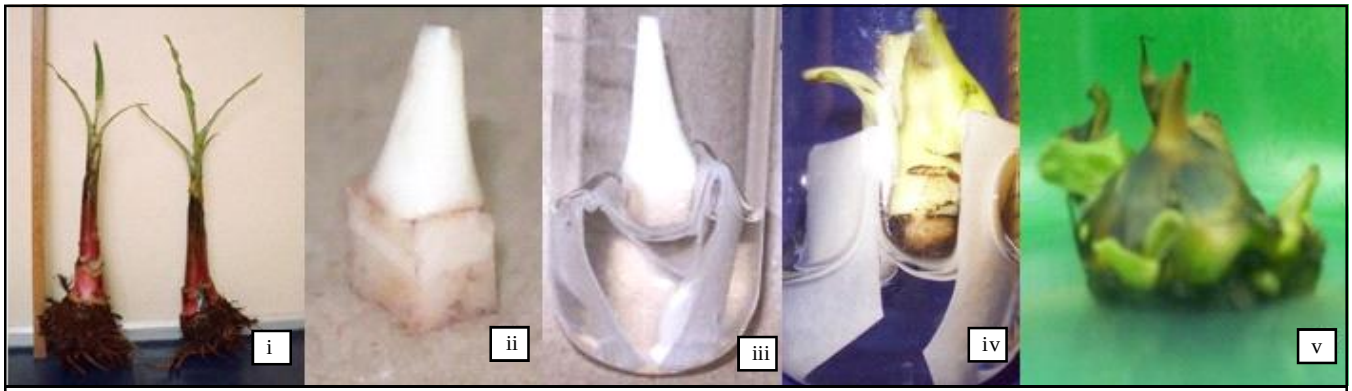

Fig. A: Initiation of aseptic culture by shoot-tip culture: (i) Sword suckers; (ii) Shoot-tip; (iii) and (iv) Shoot-tip culture: (v) Aseptic culture

explants were observed for their bulging in the tips and morphogenetic activity. Such explants were counted and expressed and in terms of per cent establishment. The successfully established cultures (Fig. A v) were excised into 2-4 sections by giving vertical cuts through the tip. The excised sections were used to carry out experiments.

Various cheaper sources of carbon such as laboratory grade sucrose (Qualigens Fine Chemicals, Navi Mumbai, India), common grade sugar (Heritage Foods (India) Ltd., Hyderabad, India), cube sugar (Daurala Sugar Works, Daurala, Meerut, India), rock sugar (Big bazaar, BSK IIIrd Stage, Bangalore, India), candy sugar (Big bazaar, BSK IIIrd Stage, Bangalore, India), glucose (Titan Biotech Limited, Biwadi, Rajastan, India), jaggery (Heritage Foods (India) Ltd., Hyderabad, India) each at 20, 30, 40 and 50 g/lit and sugarcane juice (Cane-O-La, BSK IInd Sage, Bangalore, India) at 100, 150, 200 and 250 ml/lit were added to the media and culture response was compared with analytical (AR) grade sucrose 30 g/lit (Titan Biotech Limited, Biwadi, Rajastan, India).Cultures were subcultured for 2 cycles each of 4 weeks duration. The observations on shoot and root characters were recorded at the end of second subculture cycle and after four weeks of inoculation, respectively. Further, cost per plantlet was estimated. The experimental data was statistically analyzed in a Completely Randomized Design by adopting analysis of variance technique. The levels of significance used for F test was at 1 per cent probability. Critical difference values (C.D.) values were given in the table was at 1 per cent level of significance, where the F test was significant and used to compute the means. Values in percentages were subjected to arcsin transformation to ensure homogeneity.

RESULTS AND DISCUSSION

In the present study media supplemented with two grades of commercial sugars, rock sugar 30 g/lit and common grade sugar 30 g/lit were found superior for shoot multiplication and *in vitro* rooting, respectively (Table 1-2 and Fig. 1-3). This may be probably due to their efficient translocation and assimilation by the explants resulting in enhanced cell division and eventual growth. Similar findings were also reported in banana (Ganapathi *et al*., 1995; Kodym and Zapata- Arias, 2001; Saeed, 2006 and Das and Gupta, 2009), ginger (Sharma and Singh, 1995), anthurium (Prabhakara, 1999), strawberry (Kaur *et al*., 2005) and *Centella asiatica* (Raghu *et al*., 2007). Demo *et al*. (2008) reported that the locally available sugars at 0.30 per cent enhanced proliferation of plantlets of potato similar or better than laboratory grade sucrose. On the contrary, commercial grade sugar proved inferior to all other carbon sources for micropropagation of *Wrightia tomentosa* (Joshi *et al*., 2009). They reported that the best shoot multiplication rate could be achieved on the medium containing sugar cubes as a carbon source, replacing AR grade sucrose.

Goel *et al*. (2007) observed better growth performance of *Rauwolfia serpentina* with ordinary market sugar as well as in Daurala sugar cubes. They also reported that by using market grade sugar in glass bead supported liquid medium, upto 94 per cent reduction in the medium cost was achieved. However, in the present study sugar cubes, laboratory grade sucrose, sugar candy and glucose were found inferior in their performance as compared to AR grade sucrose and rock sugar for shoot multiplication and AR grade sucrose and common grade sugar for rooting (Table 1-2).

All the carbon sources at higher concentrations reduced the shoot and root growth. This may be due to inhibitory action of higher levels of sugars as reported by Robert-Oehlschager (1988) in the culture of barley pollen with glucose. He opined that higher levels of glucose (20 g/lit) promoted early growth, but later inhibited the growth of cultures of barley pollen. Perata *et al*. (1997) showed that sugar negatively interact with signal transduction pathway of GA. It is possible that the poor growth at high concentration of carbon sources in our study is a result of repression of growth hormones such as GA in addition to its direct osmotic interference in the medium.

Glucose (30 g/lit) gave best culture response, but quality wise they were inferior as compared to AR grade sucrose or rock sugar. Previous workers have also reported preference to particular sugar and to their concentration. Debnath (2005) obtained more vigorous shoots and more callus in lingonberry on the medium supplemented with glucose or sucrose than those on medium with sorbital.

In the present study poor culture response was observed with candy sugar, whereas, sugarcane juice and jaggery were found unsatisfactory (Table 1-2 and Fig. 4). Prakash (1993) and Prakash *et al*. (2004) reported that the sugarcane juice adversely affected culture growth in ginger and turmeric and led to drying of leaves tips. This may be due to inhibitors already

present or formed during autoclaving. Joshi *et al*. (2009) opined that incorporation of jaggery in the medium for micropropagation of *Wrightia tomentosa* was not useful and rather it adversely affected the shoot and root growth.

Analysis of cost revealed that the rock sugar (30 g/lit) was found to be a low cost replacement for AR grade sucrose for shoot proliferation (Table 3). It reduced the cost by 95.85 per cent when compared with analytical grade sucrose. For *in vitro* rooting, common grade sugar (30 g/lit) was found cheaper alternative to analytical grade sucrose as it reduced the cost of the medium by 96.91 per cent.

It is obvious that the rock sugar (30 g/lit) and common grade sugar (30 g/lit) are cheaper carbon

Table 1 : Effect of different carbon sources on shoot multiplication of banana cv. 'GRANDE NAINE'

Sr. No.	Treatments	Number of shoots/ explant	Shoot length (cm)	Number of adventitious buds/ explants	Number of leaves/ shoot	Shoot diameter (mm)
1.	Sucrose AR grade 30 g/lit	16.80	3.93	2.95	3.92	3.49
2.	Sucrose LR grade 20 g/lit	6.70	4.18	2.85	3.06	2.39
3.	Sucrose LR grade 30 g/lit	7.60	4.97	1.25	3.51	3.14
4.	Sucrose LR grade 40 g/lit	6.00	4.05	2.50	2.77	3.44
5.	Sucrose LR grade 50 g/lit	5.40	3.78	3.20	2.31	3.55
6.	Common grade sugar 20 g/lit	6.10	4.10	1.60	2.43	2.19
7.	Common grade sugar 30 g/lit	8.60	4.13	4.90	3.10	3.09
8.	Common grade sugar 40 g/lit	8.20	4.14	2.65	2.96	3.59
9.	Common grade sugar 50 g/lit	1.50	2.95	0.50	1.75	3.53
10.	Cube sugar 20 g/lit	7.90	4.22	2.70	4.16	3.16
11.	Cube sugar 30 g/lit	8.30	3.99	2.25	3.41	3.39
12.	Cube sugar 40 g/lit	7.10	3.46	1.65	2.44	3.35
13.	Cube sugar 50 g/lit	1.75	3.03	1.70	2.37	2.23
14.	Rock sugar 20 g/lit	13.40	2.82	6.55	2.70	2.39
15.	Rock sugar 30 g/lit	14.10	3.48	3.65	3.40	3.03
16.	Rock sugar 40 g/lit	8.50	3.20	2.55	2.81	3.21
17.	Rock sugar 50 g/lit	5.95	2.97	2.25	2.29	3.44
18.	Candy sugar 20g/lit	5.45	3.78	1.55	3.08	2.37
19.	Candy sugar 30 g/lit	4.20	4.25	0.45	2.91	2.82
20.	Candy sugar 40 g/lit	5.40	3.29	1.71	2.20	3.36
21.	Candy sugar 50 g/lit	3.45	3.01	3.10	2.16	3.62
22.	Glucose 20 g/lit	7.40	3.37	3.35	2.89	2.46
23.	Glucose 30 g/lit	8.12	3.33	2.42	2.85	3.11
24.	Glucose 40 g/lit	7.30	3.29	1.90	2.75	3.15
25 .	Glucose 50 g/lit	2.85	2.49	3.30	2.20	3.17
	S.E. ±	0.77	0.16	0.37	0.15	0.11
	C.D. (P=0.01)	2.88	0.59	1.37	0.56	0.43

Table 2 : Effect of different carbon sources on *in vitro* rooting of banana cv 'GANDE NAINE'

Sr. No.	Treatments	Per cent rooting	Number of primary roots/ shoots	Root length (cm)	Number of secondary roots/ shoots	Plant diameter (mm)	Fresh weight of plant (mg)
1.	Sucrose AR grade 30 g/lit	100 (90)*	4.70	4.18	7.85	4.07	1428.75
2.	Sucrose LR grade 20 g/lit	100 (90)	3.47	2.69	3.95	3.17	900.25
3.	Sucrose LR grade 30 g/lit	100 (90)	5.25	3.34	5.70	3.63	961.55
4.	Sucrose LR grade 40 g/lit	100 (90)	4.15	3.98	3.40	3.77	984.35
5.	Sucrose LR grade 50 g/lit	100 (90)	3.30	3.14	11.27	4.24	1093.50
6.	Common grade sugar 20 g/lit	100 (90)	3.45	4.05	5.95	3.83	1148.55
7.	Common grade sugar 30 g/lit	100 (90)	6.55	4.10	8.00	3.59	1317.60
8.	Common grade sugar 40 g/lit	100 (90)	6.50	4.30	5.20	3.85	1431.75
9.	Common grade sugar 50 g/lit	100 (90)	4.25	5.71	15.60	4.40	1782.70
10.	Cube sugar 20 g/lit	100 (90)	3.40	3.72	3.80	3.29	912.15
11.	Cube sugar 30 g/lit	100 (90)	4.40	4.51	6.65	3.34	961.15
12.	Cube sugar 40 g/lit	100 (90)	3.90	4.48	5.90	3.53	1067.30
13.	Cube sugar 50 g/lit	100 (90)	3.30	4.85	17.00	4.21	1540.75
14.	Rock sugar 20 g/lit	100 (90)	4.05	2.53	3.70	3.32	767.50
15.	Rock sugar 30 g/lit	100 (90)	5.70	3.49	4.80	3.39	910.85
16.	Rock sugar 40 g/lit	100 (90)	4.30	3.99	6.95	3.76	968.55
17.	Rock sugar 50 g/lit	100 (90)	3.90	3.19	6.60	3.90	1048.55
18.	Candy sugar 20g/lit	100 (90)	3.65	2.50	3.32	3.16	691.00
19.	Candy sugar 30 g/lit	100 (90)	5.25	3.40	4.37	3.32	804.90
20.	Candy sugar 40 g/lit	100 (90)	4.60	3.74	6.35	3.54	889.25
21.	Candy sugar 50 g/lit	100 (90)	4.30	2.87	4.65	5.01	1186.60
22.	Glucose 20 g/lit	100 (90)	3.05	3.16	1.80	3.03	841.30
23.	Glucose 30 g/lit	100 (90)	4.75	3.38	4.45	3.40	944.75
24.	Glucose 40 g/lit	100 (90)	4.40	3.47	5.80	3.98	1209.70
25 .	Glucose 50 g/lit	100 (90)	2.80	3.49	3.05	4.03	972.45
	S.E. ±	NS	0.20	0.18	1.05	0.11	58.47
	C.D. (P=0.01)	---	0.76	0.69	3.92	0.44	217.14

*Figures in parenthesis indicate arcsin-transformed values NS= Non-significant

Fig. 1 : Different sources of carbon: (a) AR grade sucrose; (b) Rock sugar; (c) Common grade sugar

Fig. 2 : Multiple bud clump and microshoots obtained from medium supplemented with: (a) and (b) AR grade sucrose 30 g/lit; (c) and (d) Rock sugar 30 g/lit

Fig. 3: *In vitro* rooted plantlets obtained with medium supplied with: (a) AR grade sucrose 30 g/lit; (b) Commercial grade sugar 30 g/lit

Table 3 : Differential cost for one-litre media using different carbon sources

Carbon sources	Quantity of sugar used for each liter of medium (g)	Price of sugar / 500 g (Rs.)	Price of sugar for one litre of medium (Rs.)	Cost reduction over control (%)
Analytical grade sucrose (Control)	30	422.00	25.32	0
Laboratory grade sucrose	20	156.00	6.24	75.35
Laboratory grade sucrose	30	156.00	9.36	63.03
Laboratory grade sucrose	40	156.00	12.48	50.71
Laboratory grade sucrose	50	156.00	15.60	38.38
Common grade sugar	20	13.00	0.52	97.94
Common grade sugar	30	13.00	0.78	96.91
Common grade sugar	40	13.00	1.04	95.89
Common grade sugar	50	13.00	1.30	94.86
Cube sugar	20	32.00	1.28	94.94
Cube sugar	30	32.00	1.92	92.41
Cube sugar	40	32.00	2.56	89.88
Cube sugar	50	32.00	3.20	87.36
Rock sugar	20	17.50	0.70	97.23
Rock sugar	30	17.50	1.05	95.85
Rock sugar	40	17.50	1.40	94.47
Rock sugar	50	17.50	1.75	93.08
Candy sugar	20	19.00	0.76	96.99
Candy sugar	30	19.00	1.14	95.49
Candy sugar	40	19.00	1.52	93.99
Candy sugar	50	19.00	1.90	92.49
Glucose	20	115.00	4.60	81.83
Glucose	30	115.00	6.90	72.74
Glucose	40	115.00	9.20	63.66
Glucose	50	115.00	11.50	54.58

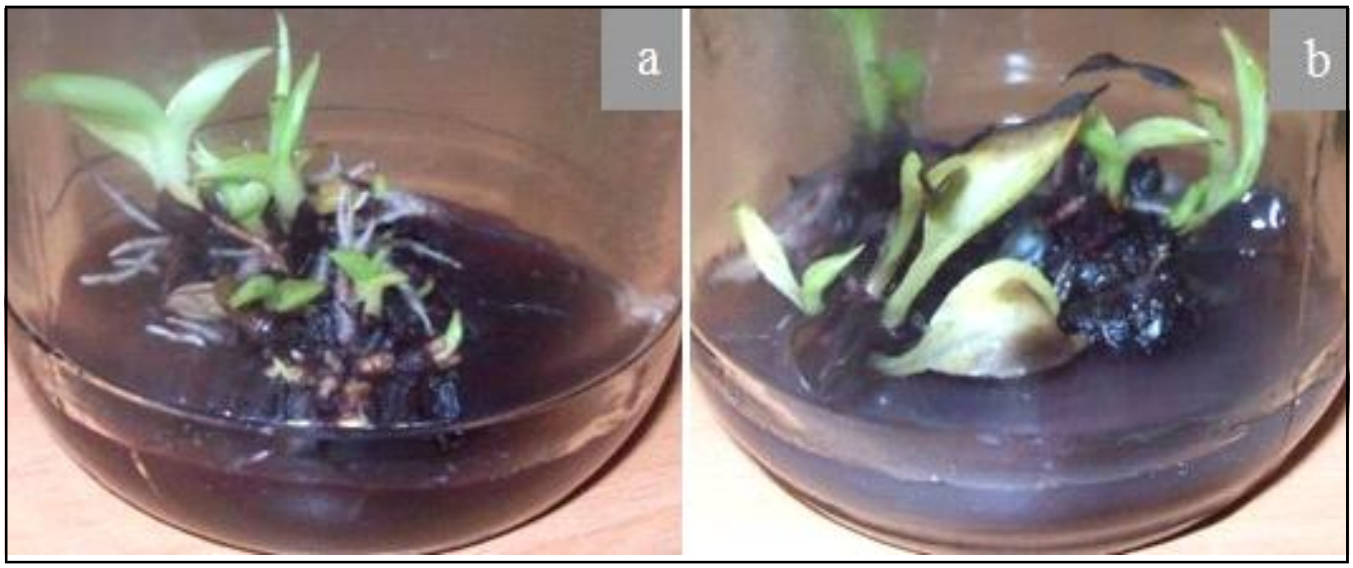

Fig.4 : Unsatisfactory culture response obtained from the medium supplemented with cheaper carbon sources: (a) Jaggery 30 g l/lit; (b) Sugarcane juice 100 ml l/lit

sources for micropropagation of banana cv. 'GRANDE NAINE'.

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