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RESEARCH PAPER

Optimization of explants density for tissue culture propagation of banana cv. 'GRANDE NAINE'

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Abstract : Cost of production is always stressed as the main obstacle for tissue culture. Nutrient media is one of the most costly input which accounts for 30-35 per cent of total cost of tissue culture propagation. Production cost, therefore, can be reduced by efficient utilization of culture media. To find out optimum quantity of media required for shoot proliferation, 1- 5 multiple bud explants were incubated in each culture bottle. Explants density of 4/culture bottles was found best as it recorded higher total shoot production/l (291.94), shoot length (3.22 cm), number of leaves/shoot (2.81) and lower cost per shoot (Rs. 1.175). *In vitro* rooting was carried out with densities of 6, 8, 10 and 12 microshoots/culture bottle. The maximum response with regard to rooting had not yet been reached as there were no significant differences among the treatments. Incubation of 4 multiple bud explants and 12 microshoots per culture bottles is optimum for scaling-up of tissue culture production of banana cv. 'GRANDE NAINE'.

Key Words : Tissue culture, Explants density, Grande Naine, MS medium, Cost/shoot

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INTRODUCTION

Edible bananas (*Musa* spp.) are among the most important food crops in the world, with a production approximating 102 million tons per year Anonymous (2002). However, expansion of banana production is limited by lack of availability of healthy and good quality planting material. The transmission of harmful insects, nematodes, viruses and disease by field grown suckers prompted interest in the use of *in vitro* techniques. However, the production cost of micropropagated plantlets is very high, making small-scale farmers reluctant to use these superior plantlets. Nutrient media is one of the most costly input which accounts for 30-35 per cent of total cost of tissue culture propagation. The production cost can be reduced by resorting to optimum explants density during micropropagation. Explant density is an important physical parameter that influences microplant growth. The need for an optimum explants density for faster micro plant growth during micropropagation has been reported in a number of crop species (Chun *et al*., 1986; Hamad and Taha, 2009; Monette, 1983 and Start and Cumming, 1976).

Although, easy to test and simple to apply, the effect of explants density on the *in vitro* multiplication of banana was totally ignored in the previous studies. In most of

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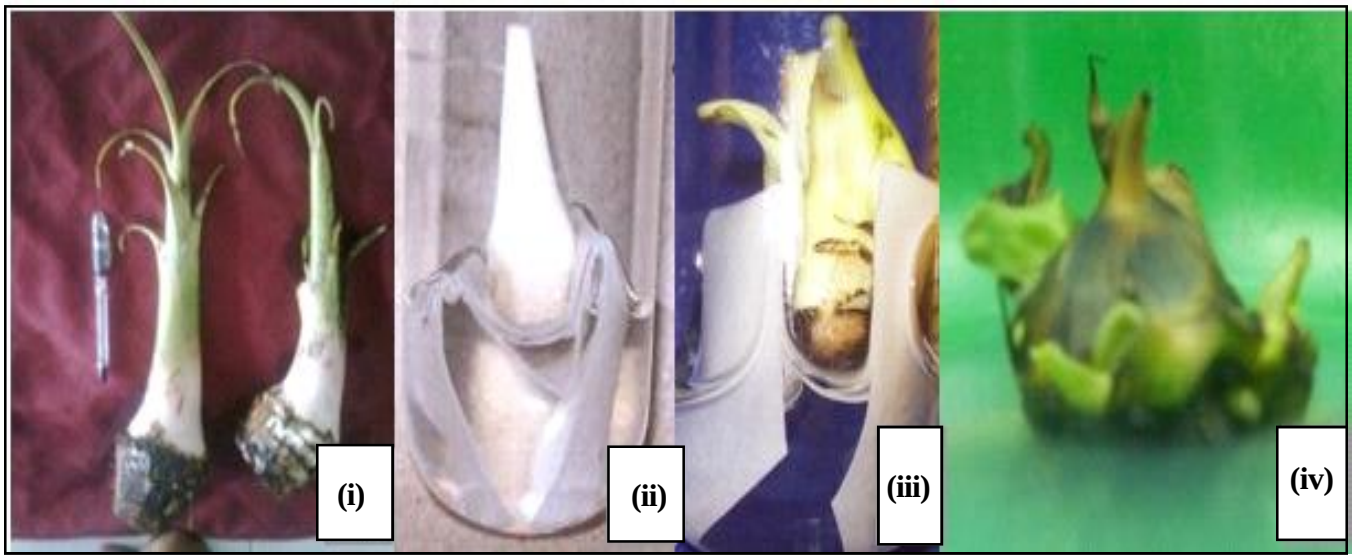

Fig. A : Initiation of aseptic culture in banana cv. GRANDE NAINE by shoot-tip culture (i) Sword suckers; (ii - iii) Shoot-tip culture; (iv) Aseptic culture

the studies, the attention was focused on optimization of hormones, carbon sources and gelling agents. Treatment with highest shoot formation/explant was considered optimal and suggested for large scale production of propagules. However, higher shoot formation/single explant is not the best indicator for commercial large scale production. The total shoot production and cost/ single shoot are the most important and essential parameters. The present study was, therefore, conducted with the objective to compare the effect of explant density on average shoot formation, shoot length, root formation, expected total shoots/l medium, the cost of single shoot and rooted plantlets.

MATERIAL AND METHODS

Healthy and vigorously growing sword suckers of banana cv. GRANDE NAINE (3-4 month), free from disease were selected as a source of explant (Fig. A i). The procedure described by Besagarahally (1996) was followed for initiation of aseptic culture with certain modifications.

Preparation of explants :

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 1 per cent (w/v) Bavistin for half an hour, to further clean the suckers. 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 0.5 per cent (w/v) Bavistin + 0.05% (w/v) 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 (w/v) cetrimide for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with 0.1 per cent $HgCl_2$ for 10 minutes. Further operations such as washing several times with sterile distilled water to remove all traces of chlorine, trimming of explant and inoculation in liquid culture media were carried out under a laminar air flow chamber.

Initiation of aseptic culture :

Shoot tip explants were incubated in MS liquid culture media containing 2 mg/lit BAP and 75 mg/lit adenine sulphate for two weeks maintaining standard culture conditions of 25 ± 2^0 C, 70 per cent RH and photoperiodic cycle of 16 hours light and 8 hours dark period (Fig. Aii-iii). After two weeks of incubation, all the explants (Fig. A iii) were evaluated for their ability to establish in liquid media. 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 semi-solid media supplemented with 2 mg/lit BAP and 75 mg/lit adenine sulphate and incubated for four weeks maintaining standard culture conditions. The explants were observed for their bulging in the tips and morphogenetic activity. The successfully established explants (Fig.A iv) were excised by trimming the discoloured tissues, then 2-4 vertical cuts were made at the tip of each explant and the used for the experiments.

Baby jar glass bottle (11 cm x 5.5 cm) each with 40 ml MS semisolid medium and multiple bud explants each having 2 to 3 growing buds were used for studies. For shoot proliferation explants density of 1, 2, 3, 4 and 5 multiple bud explants were inoculated per culture bottles. Explants were incubated for eight weeks (transferred to fresh media after four weeks) maintaining standard culture conditions. Individual microshoots were obtained by cutting the multiple shoot clumps using sterilized scalpel and cultured on rooting medium containing 2 mg/lit IBA +1 mg/lit NAA + activated charcoal 2.5 g/ l for four weeks. At the end of experiments, morphological characteristics (number of shoots/ explant, shoot length, number of leaves, roots/shoots, fresh weight etc.,) were measured. An analysis of variance (ANOVA) was conducted on data concerning shoot and root morphological parameters using the statistical programme wax vms fortran.

RESULTS AND DISCUSSION

It is evident (Table 1) that with increase in the explants density from 2 to 5/ culture bottles, the capacity of the shoot formation, shoot length and number of leaves/shoots were reduced (11.33 to 6.98; 3.77 to 2.37 cm and 3.53 to 2.29). Increasing the density of explants/ culture bottles from 3 to 5 resulted in significantly higher total shoots/litre of medium (262.44, 291.94 and 312.94) than using 1 and 2 explants/culture bottles (195.56 and 202.25).The cost/shoot was reduced from 1.754 and 1.696 rupees at density of 1 and 2 explants to 1.307 to 1.096 rupees at density of 3 to 5 explants/culture bottles.

The cost per single shoot as well as the total shoots production and rate of shoot formation per explant of 'Grand Naine' banana was significantly affected by the explants density (Table 1 and Fig. 1). The results indicated the cost/shoot could be reduced to as low as 1.307 to 1.096 rupees by incubating 4 to 5 explants/culture bottle.

Assessment based on shoot formation/explant is not enough for selection of best treatment. According to shoot formation/single explant, density of 1 explant/culture bottle would be recommended over higher density. However, compared to density of 1 explant/culture bottle, density of 4 and 5 culture bottles increased the total shoot production and reduced the cost per shoots (Table1 and Fig. 1).

Cost of production always stressed as the main obstacle of micropropagation. Nevertheless, the cost of items such as medium volume and medium use efficiency (total shoot/ litre), autoclaving and laminar operation time, labour working hours and shelving space, was not taken into consideration during assessment of different *in vitro* multiplication treatments. All these cost items are related and could be managed through selection of optimum density of explants/culture. The cost of 1 lit of medium used as a basis to compare the cost effectiveness of different cytokinins on banana shoots formation (Arinaitwe *et al*., 2000). Using the same approach Goel *et al*. (2007) reported that cost/shoot of *Rauwolfia serpentina* on semi-solid medium was Rs. 0.126 and could be reduced to Rs. 0.004 using glass beads with Daurala sugar. Similar findings were also reported by

Table 1: Effect of explants density on shoot formation in banana cv. GRANDE NAINE

Explants/ bottles (No.)	Shoots/ explants	Shoot length (cm)	Shoot diameter (mm)	Number of leaves/shoot	Fresh Wt. of shoots (mg)	Total shoots/ litre medium	Cost/shoot (Rs.)
1	21.90	3.59	4.00	3.08	641.25	195.56	1.754
2	11.33	3.77	3.75	3.53	612.50	202.25	1.696
3	9.80	3.41	3.50	3.00	551.50	262.44	1.307
4	8.18	3.22	3.00	2.81	357.00	291.94	1.175
5	6.98	2.37	1.77	2.29	291.25	312.94	1.096
C.D.(P=0.01)	7.14	0.65	1.03	0.45	66.26	116.62	----

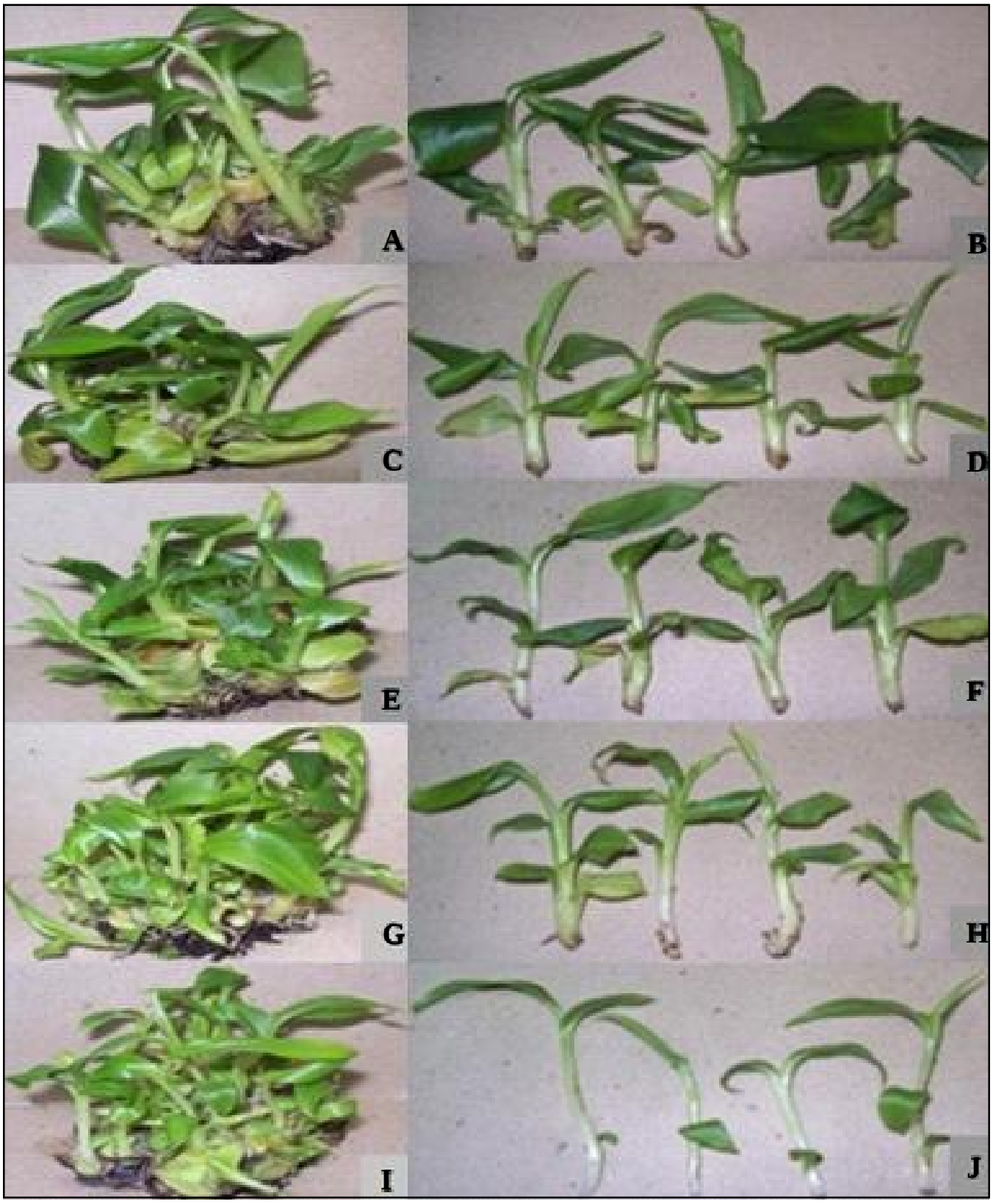

Fig. 1 : Multiple bud clump and microshoots obtained with (A) – (B) 1 explant/culture bottles; (C) – (D) 2 explants/culture bottles; (E) – (F) 3 explants/culture bottles; (G) –(H) 4 explants/culture bottles; (I) –(J) 5 explants/culture bottles

Fig. 2 : ***In vitro*** **rooted plantlets obtained with (A) 6 microshoots/culture bottles; (B) 8 microshoots/culture bottles; (C) 10 microshoots/culture bottles; (D) Twelve microshoots/culture bottles**

Table 2: Effect of microshoot density on root formation in banana cv. GRANDE NAINE

Number of microshoots/ bottles (No.)	Number of primary roots/shoots	Root length (cm)	Number of secondary roots/shoot	Plant diameter (mm)	Fresh weight of plants (mg)	Cost/ plantlets (Rs.)
6	5.29	4.77	10.08	3.35	2132.50	2.285
8	5.69	4.69	10.37	3.10	1827.50	1.714
10	4.87	4.66	13.40	3.78	2282.50	1.371
12	4.98	3.80	7.23	4.47	1547.50	1.143
C.D.(P=0.01)	1.97	1.45	5.38	1.40	770.56	-----

Hamad and Taha (2009), who observed that the cost/ shoots of pineapple could be reduced from 0.13 ringgit to 0.10 ringgit (Malaysian currency).

Maximum number of secondary roots/shoots and fresh weight/plantlets were observed when there were 10 microshoots/culture bottles (Table 2 and Fig. 2). However, the maximum response with regard to root characters had not yet reached. Therefore, more than 12 microshoots/ culture bottles might produce a greater response.

The shoot and root growth during the micropropagation appears to depend on explant density. The enhancing effect of inoculation density on shoot and root growth has been reported in asparagus (Matsubara, 1973 and Matsubara and Clore, 1974) and potato (Sarkar *et al*., 1997). This effect may be due to some growth promoting substances diffusing from the explants. There are several other factors which also influence the proliferation rate in culture. These include the explant to medium ratio, the explant to air ratio, the explant to container volume ratio and type of container closures (McClelland and Smith, 1990). The explant to medium ratio would be responsible for influencing the microplant proliferation rate in banana micropropagation.

Conclusion :

The cost of the *in vitro* shoot production of 'Grand Naine' banana could be reduced by 33 per cent by culturing at a density of 4 multiple bud clump/culture bottles. Also cost of rooting could be lowered by 50 per cent at density of 12 microshoots/culture bottle. It is evident from the present study that the incubation of 4 multiple bud clumps and 12 microshoots in each culture bottles is optimum for scaling-up of tissue culture production of banana cv. 'GRANDE NAINE'.

The calculated cost included only the variable items of cost (MS salts, sucrose, hormones, electricity cost of autoclave, laminar operation and incubation room and wages of labour)

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