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FACTORS AFFECTING *IN VITRO* SHOOT REGENERATION IN BANANA CV. GRAND NAINE

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SUMMARY

Young suckers of banana with diameter 8-12 cm were most suitable for shoot tip culture and gave maximum shoot proliferation and least browning due to phenols. Mercuric chloride (0.1%) proved better surface sterilant than ALA bleach (50%) for higher explant survival percentage (52.84%) and lower contamination per cent. Twelve minutes time for exposure of shoot tips to mercuric chloride (0.1%) was optimum for surface sterilization. Pre-sterilization of explants with 0.1 percent bavistin for 15 minutes before surface sterilization, reduced the incidence of fungal contamination. Addition of cefotaxime 500 ppm to the culture medium reduced systemic bacterial contamination. The explants cultured in the month of April, exhibited the highest establishment (67.53%). The MS medium supplemented with BAP (3 mgl^{-1}) induced maximum shoot regeneration (91.53%).

Key words: Banana, cefotaxime, plant regeneration, sterilization

INTRODUCTION

The development of micropropagation techniques has been a major focus of *Musa* research during the past two decades (Israeli *et al.* 1995). Mass propagation of banana through tissue culture has great potential for obtaining disease-free and true-to-type uniform planting material. It is possible to obtain large number of *in vitro* plantlets from one original explant within a year (Bhatt and Bhatt 2003). However, successful in *vitro* culture is not independent of several problems associated with establishment of banana cultures *in vitro.*

For *in vitro* propagation of banana, bacterial contamination is a great problem. Although initially surface sterilization works, later on microbial contamination at the base of the explant is observed within 7 to 15 days after inoculation. Bacterial growth is also observed around the explants in the culture media. Huge number of explants are destroyed in the culture due to endogenous bacteria (Hadiuzzaman *et al.* 2001). Another problem of *in vitro* cultured explants, accompanied by darkening of culture medium has been attributed to exudation of phenolic compounds from young tissue and accumulation in the culture medium. This process is initiated by browning of the surface of plant tissues due to the oxidation of phenolic compounds, resulting in the formation of quinones which are highly reactive and toxic to plant tissue. The explant establishment vis-à-vis successful *in vitro* culture of banana depends on number of factors. Therefore, in the present study the key factors such as size and type of explant, sterilization techniques to establish contamination free culture, season of explant collection, effect of antibiotics and the selection of appropriate growth regulator levels to achieve successful *in vitro* regeneration was investigated.

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MATERIALS AND METHODS

Banana (*Musa* spp.) cultivar Grand Naine, was utilized as the source material for obtaining shoot tips. Healthy sword suckers of different diameters (2-12 cm) were used as an explant in this study. Suckers were carefully removed from field, brought to the laboratory and were washed in running tap water for 15 min. The shoot tips, containing several sheathing leaf bases enclosing the axillary buds and subjacent corm tissue and measuring about 2.5-3.5 cm in length, were isolated. After preparation, the explants were washed with diluted teepol 1-3 min. The effect of explant and season/month of explant collection on the culture establishment and shoot regeneration was studied.

The explants were surface sterilized under aseptic conditions with 0.1 % mercuric chloride or ALA bleach (50%) for different durations (5-15 min.). The treated explants were then rinsed thrice with sterile distilled water before inoculation on establishment media. The suitable surface sterilant and the time required for effective surface sterilization was standardized. The surface sterilant solution was prepared fresh every time.

Surface sterilized explants (shoot tips) were inoculated in test tubes/jars containing culture medium (MS medium fortified with growth regulators and/or cefotaxime at different concentrations). The brown parts of the shoot tips were removed before inoculation to remove the toxic effects of mercuric chloride. After inoculating the explants on culture medium, the culture vessels were incubated at 25±2°C in 16 h continuous florescent light (2,000 lux) followed by dark period of 8 h. The incubation temperature and photoperiod were similar in all the experiments. The data was recorded on the per cent contamination, type of contamination, survival of explant and established cultures and percent shoot regeneration was recorded after four weeks of inoculation. Twenty cultures in each concentration formed one replication. There were three replications per treatment. The data were analyzed according to completely randomized block design (CRD) as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Size and type of explant: Young suckers with diameter 2-4 cm gave minimum shoot proliferation as compared to suckers with diameter ranging from 8-12 cm (Table 1). This may be due to the several older sheathing leaf bases enclosing the axillary buds which regenerated into multiple buds (Doreswamy *et al.* 1983). Vigourous growth and high rate of multiple shoot development was observed in shoot tips isolated from three-month-old suckers as compared to one-month-old sucker, where growth was slow coupled with low rate of multiple shoot development (Radhadevi and Nayar 1993). Extent of browning was also correlated with the diameter and/or age of the suckers. Young suckers lead to higher extent of browning as compared to the older ones.

Table 1. Effect of sucker diameter (cm) on shoot proliferation and extent of browning in banana cv. Grand Naine.

Diameter of suckers	Extent of browning	Shoot proliferation
2-4 cm	++	+
4-6 cm	++++	++
6-8 cm	+++	+++
8-10 cm	++	++++
10-12 cm	+	++++

high ++++, medium +++, low ++, very low +

Effect of surface sterilants and duration of exposure on per cent contamination and explant survival: There was a significant reduction in per cent contamination with an increase in duration of exposure to the $HgCl_2$ (0.1%) (Table 2). Among various concentrations and durations of treatment in different sterilants tried, the treatment of $HgCl_2$ for duration of 12 min was found to be the best with 52.84 % survival of uncontaminated explants, which was closely followed by 10 min duration treatment (52.50%). The survival per cent was significantly reduced at lower and higher time duration, which was 17.53 % and 33.50 % at 5 and 15 min, respectively. The use of ALA bleach as sterilizing agent at 50 % concentration

Table 2. Effect of surface sterilants on percent contamination and explant survival in shoot tips of banana cv. Grand Naine.

Duration of exposure (min)	Contamination (%)	Explant survival (%)
Control	100 (89.96)*	0.00 (0.00)
$HgCl_2$ (0.1%)		
5	81.62 (64.59)	17.53 (24.73)
10	62.02 (51.94)	52.50 (46.51)
12	55.63 (48.21)	52.84 (46.61)
15	35.69 (36.66)	33.50 (35.34)
ALA bleach (50%) **		
5	84.80 (67.02)	16.62 (24.04)
10	69.89 (56.70)	10.67 (19.05)
CD(0.05)	**1.00**	**1.28**

*figures in parentheses are the transformed values, **sodium hypochloride 4%, sodium hydroxide 1% and amine oxide 1%

gave poor results, where survival was 16.62 % and 10.67 % for 5 and 10 min duration.

The effectiveness of mercuric chloride for sterilization of banana shoot tips have also been reported by Josekutty *et al* (2003) and Sudhavani and Reddy (1999). The data further revealed that the contamination in shoot tips at 10 min. duration of exposure to $HgCl_2$ (0.1%) was 62.02 % as compared to the contamination at 12 min. (55.63%) (Table 2). The contamination percentage was reduced further (35.69%) with increase in the duration of exposure (15 min.), but the survival (33.50%) was also reduced significantly. The reduction in explant survival percentage with increase in the duration of exposure might be due to the phytotoxicity caused by mercuric (Hg^{2+}) ions present in mercuric chloride (Josekutty *et al.* 2003). Contamination rate was often higher when explants were excised from field grown suckers. To reduce the incidence of fungal contamination, the explants were treated with 0.1 % bavistin for 15 min before surface sterilization with mercuric chloride (0.1%). The pre-sterilization treatment with 0.1 % bavistin for 15 min increased the asepsis of explant. The survival percentage of explants was increased from 52.84 % to 55.43 % after pre-sterilization treatment with bavistin.

The effectiveness of antibiotic cefotaxime on per cent contamination and shoot regeneration was also studied (Table 3). The per cent uncontaminated explants were reduced with the increase in the concentration of antibiotic, but the shoot regeneration was also reduced significantly. Among the different concentrations tried, 900 ppm cefotaxime resulted in maximum number of uncontaminated culture with least shoot initiation. Maximum shoot initiation during establishment was observed under control, followed by 200 ppm and 500 ppm of cefotaxime. Delay in shoot regeneration at high concentration of antibiotic may be due to the phytotoxic effect. Elimination of bacterial contaminants by the use of antibiotics in *Musa* species has also been reported by Van den Houwe and Swennen (2000).

Table 3. Per cent uncontaminated cultures and shoot regeneration after cefotaxime antibiotic in medium during explant establishment phase.

Concentration of antibiotic (ppm)	Per cent uncontaminated cultures	Shoot regeneration
Control	45.64 (42.48)*	++++
Cefotaxime		
200	55.57 (48.17)	+++
500	65.11 (53.77)	+++
700	72.70 (58.47)	++
900	82.50 (65.22)	+
CD (0.05)	**1.01**	

*figures in parenthesis are the transformed values, high ++++, medium +++, low ++, very low +

The data clearly indicate that the maximum established cultures (67.53%) were achieved in the month of April. It decreased significantly in subsequent months and reached a minimum level (0%) in month of July (Fig. 1). The maximum percentage of culture establishment in April might be due to less contamination as compared to in the months of June and July. The high contamination rate observed during the wet season may have resulted from low

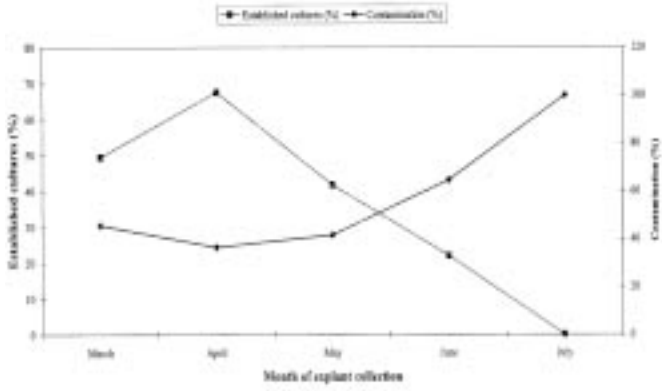

Fig. 1. Effect of month of explant (suckers) collection on established cultures percentage

temperature and high soil moisture, which is conducive for the growth of fungi and bacteria. Furthermore, endogenous phenol level, in general remains low during the month of April. Interestingly, no explant establishment was recorded in the month of July. Similar observations were reported earlier by Josekutty *et al.* (2003). On an average, the survived explants change its colour from creamy white to green within 7 to 14 days (Fig. 2). Likewise Cronauer and Krikorian (1984) and Wong (1986) also reported similar trends in shoot tip cultures of *Musa* spps. During the month of April and May, the shoot tip turned green within a week as compared to the other months (data not shown). However, in the month of November and December, maximum days (15-18) were taken by the shoot tip to turn green.

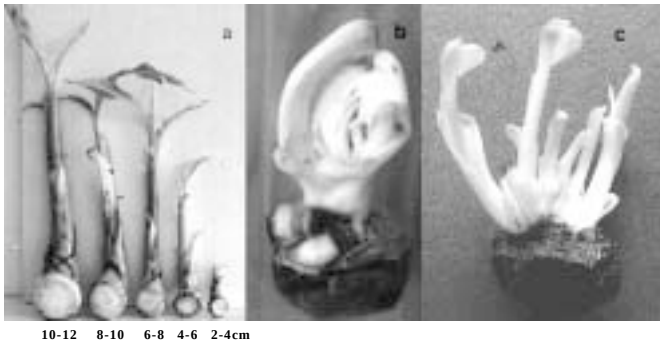

Fig. 2. Suckers of different diameters (cm) (a), shoot tip showing swelling after 7 days of inoculation (b) and shoot proliferation on MS medium fortified with BAP 3 mgl^{-1} after 60 days of inoculation (c)

The shoots from the established explants were cultured on MS medium supplemented with varying levels of BAP and IAA. The maximum mean shoot regeneration (91.53%) was observed in MS + BAP (3 mgl^{-1}) medium, which was at par (90.38%) with MS + BAP (3 mgl^{-1}) + IAA (0.1 mgl^{-1}) medium. While the minimum mean shoot regeneration (24.45%) was recorded in MS basal medium (Fig. 3). In general, MS medium containing BAP has been frequently reported to induce better shoot multiplication than other cytokinins for micropropagation of many banana varieties (Cronauer and Krikorian 1984).

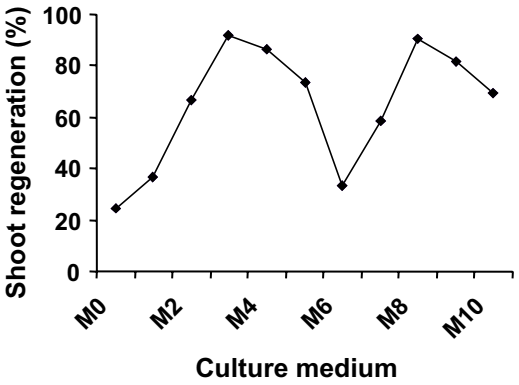

Fig. 3. Effect of medium composition on percent shoot regeneration. M0: MS Basal (control), M1: MS+BAP (1 mgl^{-1}), M2: MS+BAP (2 mgl^{-1}), M3: MS+BAP (3 mgl^{-1}), M4: MS+BAP(4 mgl^{-1}), M5: MS+BAP(5 mgl^{-1}), M6: MS+BAP (1mgl^{-1}) + IAA (0.1 mgl^{-1}), M7: MS+BAP(2 mgl^{-1})+ IAA (0.1 mgl^{-1}), M8: MS+BAP(3mgl^{-1})+IAA (0.1 mgl^{-1}), M9: MS+BAP(4mgl^{-1})+ IAA(0.1 mgl^{-1}), M10: MS+BAP(5mgl^{-1})+ IAA(0.1 mgl^{-1})

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