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***In vitro* Multiplication of Banana c.v. Desi**

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Abstract: *In-vitro* multiplication protocols of banana cultivar Desi were studied. It was observed that 6-Benzylamino purine (BAP) played a vital role for shoot multiplication. BAP at 5 mg/l resulted in maximum (4 to 5) plantlets. It was also observed that kinetin at 2 mg/l enhanced shoot elongation. Secretions of phenolic compounds were controlled by the addition of 100 mg/l tyrosine.

Key words: *In vitro* multiplication, 6-Benzylamino purine, kinetin, tyrosine

Introduction

Banana (*Musa* spp.) are seed sterile and parthenocarpic in nature and are normally propagated by suckers. In this method the rate of multiplication is highly limited even though, several attempts were made for maximising the multiplication of planting materials through various system under *in vivo* conditions (Ascenso, 1967; Baker, 1959; Hamilton, 1965). However, attention has been drawn to the possibility of using aseptic culture techniques, which facilitate quick multiplication in higher quantity.

Banana culture in Pakistan especially in the Sindh province is under decline because of the recent but rapid spread of the Banana Bunchy Top Virus (BBTV). This disease is emerging as a major threat to the cultivation of banana because no resistant cultivar to this disease is known to exist in this country. Banana is also cultivated in Northern Punjab at a very limited scale due to low sucker production and severe climatic conditions particularly during winter. As Banana Bunchy Top Virus (BBTV) is present in Southern Pakistan which may travel to the upper Punjab through some vector or planting material transported from Sindh resulting in a serious destruction of banana in this area. Thus, at present availability of disease free plants has become one of the major problems for banana cultivation. For the renaissance of this crop, rapid multiplication of disease free plants seems to be one of the top priorities in the cultivation of this important fruit crop. Under present situation, new strategies including implementation of biotechnologies appear the most suitable approach to achieve this goal. Advances in the *in-vitro* culture methodologies i.e., micropropagation and their application have made significant contribution (Cronauer and Krikorian, 1984b; Doreswamy and Sahijram, 1989; Drew *et al.*, 1992; Gardner, 1993; Gupta, 1986; Jarret *et al.*, 1985; Khatri *et al.*, 1996; Siddiqui *et al.*, 1991) to resolve these challenging problems. Micropropagation of banana through shoot tip has lead to rapid multiplication and virus elimination (Drew *et al.*, 1992). The present study was planned to establish a micropropagation system for the banana cultivar 'Desi' popularly grown in Punjab.

Materials and Methods

The work has been developed with 'Desi' cultivar collected from different sectors of Islamabad/Rawalpindi. Two weeks old sword banana suckers attached to fruiting mother plants were used as a source of shoot-tips and served as the primary explants. Mother plants were carefully inspected to make sure

that they were healthy and phenotypical true-to-type. After separating the suckers from the mother plant, their leaves along with leaf sheaths were removed with a knife and shoot tips of size 5 mm (Gupta, 1986; Jarret, 1986a, b; Novak *et al.*, 1986; Krikorian and Cronauer, 1984; Sandoval, 1985) were finally excised (Fig. 1). These shoot tips were then surface sterilized by immersion in sodium hypochlorite (2.75%) solution added with few drops of liquid soap (Zip) for 10 minutes and rinsed thoroughly with distilled autoclaved water.

Media used for culture initiation: Basal Medium MS Salts + Vitamins + 100 mg/l Tyrosine + 160 mg/l AdSO₄ + 30 mg/l Sucrose.

For culture initiation medium as described by Khatri *et al.* (1997) was used with some modification.

B.M + 1.0 mg/l BAP + 0.1 mg/l IAA.

Media used for multiplication: For multiplication, same basal medium was used with different hormones as given in Table 1.

Table 1: Media used for multiplication

Basal Medium	Treatments IAA (mg/l)	BAP (mg/l)	Kinetin (mg/l)	
B.M +	T ₀	0.0	0.0	0.0
	T ₁	5.0	0.0	1.0
	T ₂	5.0	2.0	1.0
	T ₃	0.0	2.0	1.0

The pH of medium was adjusted at 5.7 prior to autoclaving. The medium was dispensed into 250 ml conical flasks capped with cotton plugs and autoclaved at 121°C for 15 minutes. The culture were incubated at 25 ± 2°C on a rotatory shaker at 50 rpm under a 16 hours photoperiod. Eleven replicates for each treatment were used.

Results and Discussion

Establishment Phase: Results of sterilization are shown in Table 2. Explants were surface sterilized before transferring to culture medium. For sterilization normally sodium hypochlorite was used at different concentrations ranging from 0.5 to 5.25% (Cronauer and Krikorian, 1984a, b; Jarret *et al.*, 1985; Sandoval, 1985; Damasco and Barba, 1984, 1985). In the present study 2.75% sodium hypochlorite

Table 2: Effect of surface sterilization on explants of banana

No of Survival Cultures	Contaminated	Contamination %age	Survived %age
50	6	12	44
			88

Table 3: Rate of multiplication on four different media for banana regeneration through shoot tip culture

Treatments	No. of Culture	No. of Shoots/explant	
		Range	Mean
T ₀	11	1	1
T ₁	11	4-5	4.45
T ₂	11	1-2	1.27
T ₃	11	1	1



Fig. 1



Fig. 2



Fig. 3



Fig. 4

Fig. 1-4: (1) Culture initiation on liquid medium. Fig. 2, 3 and 4 shoot multiplication on T₁ T₂ and T₃ respectively

was used. Survival %age of 88 was observed, which is quite reasonable.

Shoot tips were established on liquid MS medium with 1.0 mg/l BAP and 0.1 mg/l IAA. Plain MS medium was used as control on which no response was observed. The colour of

survived explants changed from creamy white to green after 21 days of inoculation. Anthexplants grew to 10-15 mm. The banana plants have an excess of polyphenolic compounds and the explant excretes these polyphenols *in vitro* which caused blackening of the media and explant. This ceased the growth and ultimately the explant died. To control the polyphenolic compounds different treatments were given to explants i.e., antioxidants like citric acid and ascorbic acid (Mante and Tepper, 1983). In present study these polyphenolic secretions were controlled by immersion of explant in antioxidant solution made from 0.4 mg/l citric acid and 0.2 g/l ascorbic acid prior to sterilization and by the addition of 100 mg/l tyrosine in the medium as described by Hwang *et al.* (1984). It was also observed that degree of blackening of the culture medium was considerably reduced by early transfer of culture to fresh medium.

Multiplication Phase: Multiplication results are shown in Table 3. After three weeks of culture initiation explants were shifted to multiplication medium. The composition of multiplication media is given in Table 1. The oxidized part of explant was removed before transferring to multiplication medium. Meristematic appearances were seen at the base of the explant under the first leaf indicating the apical dominance by BAP after 4 weeks. These meristematic tissues appeared in shootlets after further two weeks. Maximum multiplication rate on T₁ (5 mg/l BAP) was observed, which was about 4-5 shoots/explant (Fig. 2). Similar BAP concentration was used for Musa multiplication by Khatri *et al.* (1997). Cronauer and Krikorian (1984b), Jarret *et al.* (1985) and Vuylsteke and De Langhe (1985) but the multiplication in present case was much lower which could be attributed to cultivar specificity. Presumably, variation in multiplication rate is due to different responses to the cytokinin concentration in the medium. Krikorian and Cronauer (1984) have suggested that differences in sucker production *in vivo* may also overcome *in vitro* conditions but this is not reported by other workers. When BAP along with kinetin was used T₂ (5 mg/l BAP + 2 mg/l Kinetin) the rate of multiplication was about 1 to 2 shoots/explant. The multiplication rate may have decreased due to cumulative effect of BAP along with kinetin, but the shoot elongation in this treatment T₂ (5 mg/l BAP + 2 mg/l Kinetin) was more as compared to T₁ (5 mg/l BAP). This higher growth rate seems to be due to less number of shoots and diversion of nutrients from cell multiplication towards elongation as shown in (Fig. 3) when only kinetin T₃ (2 mg/l) was used there was no multiplication but the rate of growth was better (Fig. 4) as compared with T₁ (5 mg/l BAP) and T₂ (5 mg/l BAP + 2 mg/l Kinetin).

Plain MS medium was used as control (T₀) on which no shoot multiplication and shoot elongation was observed, indicating that hormones are necessary with other nutrients for shoot multiplication and shoot elongation. It is concluded from this study that BAP played a vital role in enhancing shoot multiplication and kinetin in the shoot elongation of banana.

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