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In vitro shoot multiplication in different species of Banana

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ABSTRACT

The present investigation was undertaken to study the effect of different concentrations of BAP on shooting in different species of Banana viz. Ardhapuri, Basrai, Shrimanti. Sword suckers with medium size were inoculated on M. S. medium supplemented with different concentrations of BAP (3mg/l, 5mg/l, 7mg/l, 9mg/l) cultures were incubated at 25±1^0 C with a 16 hr photoperiod (2000 lux) provided by cool white florescent tubes. The pH of medium was adjusted to 5.8 prior to autoclaving. The materials were sub-cultured at 30 day interval in same medium to produce multiple shoots. The effect of different concentration of regimes of BAP on bud initiation and shoot multiplication were investigated.

Key words: Banana, *in vitro* shooting, BAP.

INTRODUCTION

Banana originated from the South East Asian region, where the greatest diversity of edible bananas are found [14]. Bananas account for approximately 22% of the fresh fruit production and are ranked as the second most important fruit crop [10]. The different types of banana have not been fully exploited since Cavendish types mainly dominate the market place globally. For commercialization, it is important that consistent supplies of good quality bananas are produced. This could be achieved through clonal planting materials obtained through tissue culture propagation technique. This technique provides high rates of multiplying genetically uniform, pest and disease-free planting materials. Propagation of banana through *in vitro* techniques has been reported by several workers using different explants sources and methods [7, 15, 20, 17, 18, 12, 13.]. In tissue culture, plant growth regulators (PGR) are critical media components in determining the developmental pathway of the plant cells. Cytokinins such as benzylaminopurine (BAP) and kinetin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana [7]. The most established banana shoot-tip culture system was achieved by using BAP as a supplement to

basal media [8]. The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas [3, 6, 11, 12]. BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures [1, 3]. Meanwhile, combinations of BAP with auxins such as indole acetic acid (IAA) or indole-3-butyric acid (IBA) were also used for *in vitro* multiplication of bananas [4, 12]. Although BAP stimulates shoot proliferation in bananas, it is also known to have mutagenic effects at high concentration producing off type plantlets [2]. Appearance of the off-type plantlets from *in vitro* multiplication process is considered a great disadvantage. The purpose of this study is to develop a regime for the use of BAP that is widely used to increase the multiplication rate for plantlets and concurrently control its mutagenic effect so as to decrease the percentage of morphologically abnormal plantlets formation.

MATERIALS AND METHODS

Plant materials

Sword suckers with medium size were carefully removed from field grown banana plant. The older leaves were excised with stainless steel knife. The shoot tips about 3-4 cm length were excised, each having meristem, young leaves and node. These shoot tips were finally brought to the size of 5-8 mm with the base and shoot apex. The shoot tips 3-4 cm length were excised each having meristem, young leaves and node. They were washed thoroughly with a solution of Tween - 80. (2-3 drops in 500 ml water). All traces were removed by repeated washings under running tap water for 4-5 times and finally with distilled water. These shoot tips were treated with 0.1 percent HgCl2 Solution for 7 minutes. The shoot tips were rinsed with sterile distilled water under aseptic conditions. All explants were placed on MS medium with different concentration of BAP as shown in the table no. 1

Table 1: Different treatments used for shooting

Treatments	Mg/L
MS + BAP	3
MS + BAP	5
MS + BAP	7
MS + BAP	9

All cultures were incubated at 25 ± 1^0 C with a 16 hr photoperiod (2000 lux) provided by cool white florescent tubes. The pH of medium was adjusted to 5.8 prior to autoclaving. The materials were sub-cultured at 30 day interval in same medium to produce multiple shoots.

RESULTS AND DISCUSSION

The results of multiple shoot development, growth of development, mean number of multiple shoots developed, and effect of different concentration of BAP on induction of in different genotype are presented in table no. 2.

The effect of different concentration of regimes of BAP on bud initiation and shoot multiplication were investigated. After the initial bud was sub-cultured, multiple adventitious buds were produced from the base of the explants after 30 days. Table no. 2 shows the number of

multiple shoots developed at different levels of BAP in variety Basrai. The frequency of bud formation doubled and the fresh weight increased about four times higher in media with BAP at 7mg/l when compared to media supplemented with 3mg/l BAP. Table no.3 shows the number of multiple shoots developed at different levels of BAP in variety Shrimanti. The frequency of bud formation doubled in media with BAP at 5mg/l when compared to media supplemented with 3mg/l BAP. Table no. 4 shows the number of multiple shoots developed at different levels of BAP in variety Ardhapuri. The frequency of bud formation increased in media with BAP at 7mg/l when compared to media supplemented with 3mg/l BAP. Table no. 5 shows the mean number of multiple shoots developed at various levels of BAP in three different varieties of banana. 7mg/l BAP shows increased average no. of shoots on the other hand 3mg/l BAP shows least no. of shoots in all three varieties. In the present investigation we studied effect of different concentration of BAP on three cultivars of banana, similar study was also confirmed on single cultivar by various workers.

Table 2: Number of multiple shoots developed at different levels of BAP in variety Basrai, Shrimanti, Ardhapuri

Explant No.	No. of multiple shoots at different levels of BAP											
	BASRAI				SHRIMANTI				ARDHAPURI			
	3mg/l	5mg/l	7mg/l	9mg/l	3mg/l	5mg/l	7mg/l	9mg/l	3mg/l	5mg/l	7mg/l	9mg/l
1	1	2	3	3	3	5	4	3	3	4	8	6
2	2	2	6	4	1	4	3	2	3	3	7	6
3	1	5	5	2	5	1	3	3	3	2	7	3
4	1	4	4	2	3	3	1	6	3	2	5	5
5	3	3	6	5	3	2	2	3	2	2	3	4
6	2	3	2	1	2	5	3	1	3	3	4	6
7	1	2	7	3	5	3	1	1	2	4	8	2
8	1	3	4	6	2	3	1	2	1	4	8	7
9	3	2	5	2	3	5	3	3	3	1	5	5
10	2	4	3	4	3	4	2	3	3	5	7	5
Average no. of shoots/ explants	**1.7**	**3.0**	**4.5**	**3.2**	**3.0**	**3.5**	**2.3**	**2.7**	**2.6**	**3.0**	**6.2**	**4.9**

Figure.1. Multiple shoots developed at different levels of BAP in variety Basrai, Shrimanti, Ardhapuri

1. Multiple shoots developed at different levels of BAP in variety Basrai 2. Multiple shoots developed at different levels of BAP in variety Shrimanti 3. Multiple shoots developed at different levels of BAP in variety Ardhapuri

Table no. 3 Mean no. of multiple shoots developed at various levels of BAP

Media	No. of multiple shoots			Mean no. shoots/explants
	Ardhapuri	Basrai	Shrimanti	
MS+ 3 mg/l BAP	2.6	1.7	3.0	2.4
MS+ 5 mg/l BAP	3.0	3.0	3.5	3.1
MS+ 7 mg/l BAP	6.2	4.5	2.3	4.3
MS+ 9 mg/l BAP	4.9	3.2	2.7	3.6

Olivia and Barba obtained 10.10 numbers of shoots in the optimum concentration of 10.0 mg/l BAP. They also found that increasing results in the proliferation of shoots in the increase of cycles of culture (first cycle 11 .32 and 4th cycle 17.78 number of shoots). Doreswamy *et al.* [5] obtained 20-25 shoot buds and 35 shoot lets in MS + 10 mg/l + 15% CM.

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