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IN VITRO PROPAGATION OF RED BANANA *(MUSA ACUMINATA)*

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ABSTRACT
The investigation was carried out on effects of cytokinins *viz* Benzyl aminopurine (BAP) and Kinetin (Kn) in different concentrations (*i.e* 1, 2, 3 and 4 mg L^{-1}) in combination with an auxin *viz*., Indole acetic acid (IAA) (1mg L^{-1}) on shoot proliferation and the effect of auxins *viz*., Indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) in different concentrations (0.5, 1 and 1.5 mg L^{-1}) in combination with Activated charcoal (2mg L^{-1}) on rooting of Red banana *in vitro* were investigated at Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendr, Bangalore during 2011-2012. Among the treatments, MS medium supplemented with 1mg L^{-1} IAA and 4 mg L^{-1} BAP produced the highest number of shoots/explant (8.80) while the least number of shoots (2.60) was observed with MS medium without cytokinins. On the other hand the greatest number of roots (4.90) and longest roots (8.99 cm) were induced by MS medium supplemented with 1.5 mg L^{-1} IBA and 2.0 g L^{-1} activated charcoal while the least number of roots (2.80) and shortest roots (4.981 cm) were observed with MS medium + 1.0 mg L^{-1} NAA + 2.0 g L^{-1} activated charcoal + and MS medium + 0.5 mg L^{-1} NAA + 2.0 g L^{-1} activated charcoal respectively.

INTRODUCTION

Banana accounts for approximately 22 per cent of the world fresh fruit production and is ranked as the second most important fruit crop and makes 39.8 per cent of India fruit production (Anon., 2012). Propagation of banana through suckers is seriously limited due to low multiplication rate, clonal degradation and the perils of spreading disastrous diseases. Micropopagation is preferred over conventional method of propagation owing to its faster multiplication rate, uniformity in planting materials and production of disease-free materials. Although conventional method of vegetative propagation has commercial acceptability, to ensure an extremely rapid rate of multiplication, tissue culture technique has definite and indispensable advantage over the conventional method. This technique is independent of season due to controlled conditions and requires limited quantity of plant tissue as the explant source. The apical meristem or shoot-tip culture is very efficient for rapid clonal propagation (Nandi *et al.,* 1998 and Arinaitwe *et al.*, 2000). Moreover, *in vitro* propagated banana plants give higher bunch weight, more fingers and hands and less variability in fruit size and shape (Kwa and Ganry, 1989).Though abundant information on the micropropagation of banana is available for cultivars in India and abroad (Dore Swamy *et al.*, 1983; Zamora *et al.*, 1986; Sharma *et al.*, 1997 and Balakrishnamurthy and Sreerangaswami, 1987), *In vitro* propagation through shoot-tip culture does overcome these problems. Cytokinins have been reported by many authors to induce shoot proliferation. N^6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Robert *et al.,* 2013).

Many research works (Robert *et al.,* 2013; Gubbuk and Pekmezci (2004); Rahman *et al.* (2002) and Babylatha *et al.,* 1997) on *in vitro* propagation of various banana varieties have been carried out in India. However despite having geographical indication in Kamalapura, Gulbarga district, Karnataka, Red banana remains underexploited and it is hard to find any research being done to enhance the supply of quality banana planting material to the farmers with the exception being the work done by Bharat *et al.* (2012). This technique is independent of season due to controlled conditions and requires limited quantity of plant tissue as the explant source. The present study was therefore undertaken to identify the optimum concentration of cytokinin for shoot proliferation and find appropriate type and concentration of auxin for rooting of this useful variety of banana under *in vitro* conditions.

MATERIALS AND METHODS

The present study was carried out at Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendr, Bangalore during 2011-2012. Healthy and vigorous sword suckers of 2-3 months of cv. Red banana grown under field condition were selected and carefully removed from disease free mother plants in Gulbarga district, Kamalapura region of Karnataka state, India. The selection of these plants was also based on the performance of mother plants with respect to yield potential. The leaves and part of the roots were then removed with stainless steel knife and transported to UAS Bangalore. As soon as the suckers reached the laboratory, they were

thoroughly washed in running tap water followed by washing with a detergent solution (teepol 0.5%) to remove adhering soil particles. The remaining parts of the root and the top of the plant material were cut off using a sharp stainless knife. Later, rhizomes and remaining part of pseudostem (around 50 cm long) were kept immersed in a fungicide solution (Carbendazim 1 per cent w/v) for 30 minutes before further cleaning the plant material. The outer leaves and corm tissue were trimmed to expose the shoot tip of 10 cm length and 5 cm diameter and again immersed in Carbendazim 1 per cent (w/v) for 20 min and cut further to about 3 cm diameter and 5 cm length and then rinsed four times in distilled water to remove stains of Carbendazim. After trimming one more outer layer, shoot tips were immersed in 0.2 per cent Streptomycin for 15 minutes and then for 10 minutes. In between the two treatments with Streptomycin using sharp sterile blade, one to two outer juvenile leaves and the corm base were trimmed out. The shoot tips were again rinsed five times in double distilled water to remove all traces of the streptomycin. Afterwards, the shoot tips were rinsed four times in sterile water in aseptic condition (under laminar air flow) and disinfected with 0.1 per cent Mercuric chloride ($HgCl_2$) for 8 minutes. Surface sterilized shoot tips were rinsed four times in sterile water. The outer surface of explant exposed to sterilizing agent was removed and the explants trimmed using surgical blade to bring the final size to about 3 cm length and 1cm diameter. The explants were inoculated under aseptic conditions on Murashige and Skoog (MS) (1962) semi-solid culture medium supplemented with 2.5 mg L^{-1} BAP, 80 mg L^{-1} Adenine hemisulphate, and 30 g L^{-1} sucrose. The pH was adjusted to 5.8 and agar (6g L^{-1}) was added slowly to the boiling medium and mixed well till it dissolved completely. The medium was then autoclaved for 20 min at 121p C under 1.1 kg cm^2 pressure. The cultures were then incubated at 25 ± 2p C temperature, 60-70 percent relative humidity and photoperiodic cycle of 16 hours light (2000 lux) provided by cool white fluorescent tubes and 8 hours dark period for three weeks. The successfully established explants were transferred to fresh medium every three weeks. At the end of the second subculture, the clumps were transferred to multiplication medium made of MS medium supplemented with ascorbic acid (50 mg L^{-1}), IAA (1 mg L^{-1}), sucrose (30g L^{-1}), agar (6 g L^{-1}) and different concentration of BAP and KN each at 1, 2, 3 and 4 mg L^{-1} which along with control (without cytokinins added) make up 9 treatments investigated for shoot proliferation of Red banana.

The experiment was arranged in completely randomized design (CRD) with five replications; each replicate consisted of one culture bottle. At the end of culture cycle the proliferation responses (number of shoots/explants and shoot length) were recorded. The results were analyzed using analysis of variance (ANOVA).For rooting, the microshoots (3-5 cm in length with 3-4 well developed leaves) obtained from the previous experiment were transferred to fresh MS medium supplemented with sucrose (30g L^{-1}), agar (6 g L^{-1},) 2g L^{1} activated charcoal (AC) and different concentrations of IBA (indole-3-butyric acid) and NAA (naphthalene acetic acid) each at 0.5, 1.0 and 1.5 mg L^{-1}. The experiment was arranged in CRD with ten replications, each unit of two microshoots in a bottle was considered as replicate. The microshoots were incubated for three weeks maintaining standard culture conditions. The effect of different concentration of auxins, on the production of adventitious roots (number of roots, length of roots, rooting percentage) was studied. The results were analyzed using analysis of variance (ANOVA).

In vitro rooted plantlets were washed and given a quick dip in 0.1% Carbendazim and hardened on cocopeat in polytunnel located in shade house for five weeks. The acclimatization was completed by placing the plantlets in soil, sand and cocopeat (1:1:1, v/v) in green house. After six weeks the plantlets had attained a height of 25 centimetres and were transferred to the field.

RESULTS AND DISCUSSION

During the culture period, the number of shoots per explant and the length of the shoots increased gradually in all media as the experiment progressed in Table-1. The highest rate of increase was observed with the MS medium supplemented with 1 mg L^{-1} IAA and 4 mg L^{-1} BAP while the lowest rate of increase was recorded with medium without cytokinins. The medium containing 4 mg L^{-1} BAP produced the highest number of shoots (8.80) at the end of three weeks period while the medium without cytokinins produced the lowest number of shoots (2.60). Similarly MS medium supplemented with 1 mg L^{-1} IAA and 4mgL^{-1} BAP produced the longest shoots (4.360 cm) whereas medium without cytokinins produced the shortest shoots (1.72 cm) presented in Table-1. The results indicate the importance of cytokinins in shoot proliferation of banana.

Table 1: Effect of BAP and Kinetin on shoot proliferation of Red banana

CytokininsMg L^{-1}	Number of shoots per explant			Shoot length (cm)		
	After 1 week	After 2 weeks	After 3 weeks	After 1 week	After 2 weeks	After 3 weeks
MS+ 1 IAA(Control)	1.40	2.40	2.60	1.02	1.30	1.72
MS+1 IAA +1 BAP	2.40	4.00	6.00	1.36	2.04	2.66
MS+1 IAA + 2 BAP	3.00	4.60	6.20	1.56	2.30	2.98
MS+1 IAA +3 BAP	3.20	5.40	7.20	1.62	2.96	3.68
MS+1 IAA +4 BAP	3.80	6.00	8.80	2.06	3.38	4.36
MS+1 IAA +1 Kinetin	2.20	2.80	3.60	1.28	1.82	2.26
MS+1 IAA +2 Kinetin	2.80	3.40	4.40	1.44	2.24	2.76
MS+1 IAA +3 Kinetin	3.20	4.00	5.20	1.74	2.20	2.96
MS+1 IAA +4 Kinetin	3.40	4.60	5.60	1.86	2.60	3.26
SE m ±	0.290	0.376	0.332	0.131	0.152	0.144
CD at 1%	0.791	1.023	0.903	0.360	0.389	0.397

Table 2: Effect of IBA and NAA on *in vitro* rooting of Red banana

Treatments	Number of roots per plantlet			Root length (cm)		
	After 1 week	After 2 weeks	After 3 weeks	After 1 week	After 2 weeks	After 3 weeks
MS +2.0 g L^{-1}AC (Control)	1.60	2.60	3.30	2.40	4.215	6.615
MS+2.0 g L^{-1}AC +0.5 mg L^{-1} IBA	2.10	3.20	3.90	2.54	4.716	7.346
MS+2.0 g L^{-1}AC +1.0 mg L^{-1} IBA	2.40	3.30	4.70	2.61	5.535	7.711
MS+2.0 g L^{-1}AC +1.5 mg L^{-1}IBA	1.90	4.00	4.90	2.92	6.485	8.990
MS+2.0 g L^{-1}AC +0.5 mg L^{-1}NAA	1.00	2.70	3.60	2.18	3.620	4.981
MS+2.0 g L^{-1}AC +1.0 mg L^{-1} NAA	2.20	2.50	2.80	2.23	4.030	6.565
MS+2.0 g L^{-1}AC +1.5 mg L^{-1} NAA	1.70	2.90	3.70	2.74	4.625	6.670
SE m ±	0.111	0.144	0.223	0.142	0.194	0.213
CD at 1%	0.313	0.381	0.580	0.376	0.512	0.565

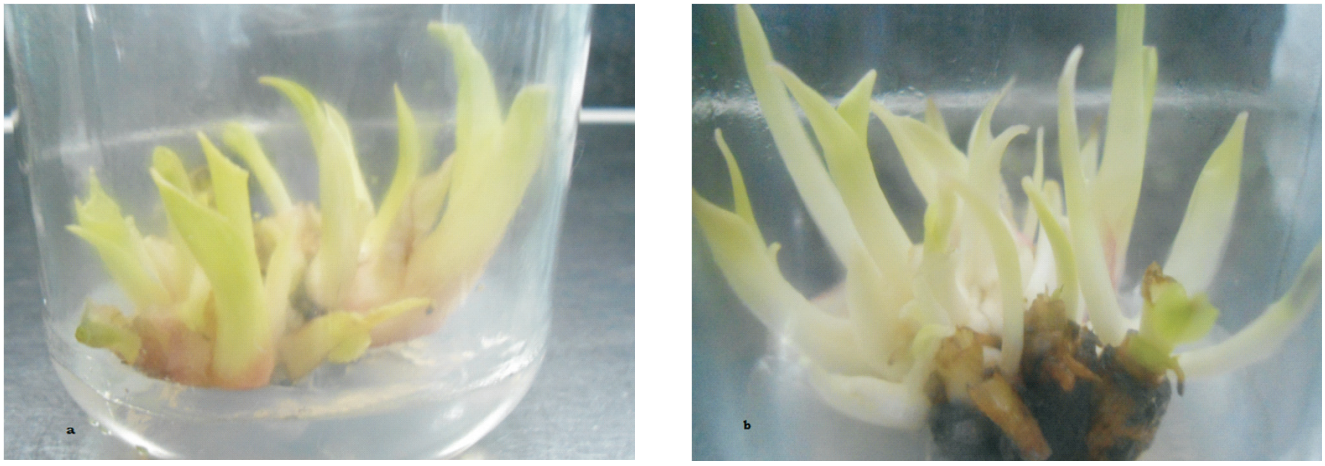

Plate 1: Shoots induced by different kind and concentration of growth regulators a) Shoots induced by 4 mg L^{-1} Kinetin, b) Shoots induced by 4mg L^{-1} BAP

High frequency proliferation in Red banana was achieved on MS medium supplemented with 2.5 mg L^{-1} BAP, 40 mg L^{-1} Adenine Sulphate, 1.5 mgL^{-1} Tyrocine and 1mg L^{-1} L-Cysteine reported by Bharat *et al.* (2012). Shoot proliferation rate and elongation is significantly dependent on cytokinin type, its concentration and the genotype of banana cultivar confirmed with results of Arinaitwe *et al.* (2000) and Gubbuk and Pekmezci (2004). The results also showed that BAP was more effective than Kinetin in terms of shoot multiplication and shoot length. The marked effects of BAP on shoot formation compared to Kinetin observed in this study may be attributed to its high stability in *in vitro* cultures (Klem *et al.*, 2004). The results of this study are in agreement with Rahman *et al.* (2002) and Buah *et al.* (2010). Rahman *et al.* (2002) reported that MS medium supplemented with 5 mg L^{-1}BAP induced 2.83 shoots while the same concentration of Kinetin induced 1.67 shoots. Buah *et al.* (2010) observed that, medium supplemented with 4.5 mg L^{-1} BAP produced an average of 13.25 shoots per explant compared to the 7.5 on the same concentration of kinetin. Anbazhagan *et al.* (2014) observed MS medium supplemented with BAP+IAA at the concentration of 3.0mg/l and 0.5mg/L was good for shoot inductions respectively.

The results observed in this experiment are also in agreement with Gubbuk and Pekmezci (2004), and Shirani *et al.* (2009) reported shoot regeneration in MS medium without cytokinins. This study also showed that BAP induced longer shoots that Kinetin which is in agreement with Rahman *et al.* (2005) and Buah *et al.* (2010). Rahman *et al.* (2005) observed that MS medium supplemented with 5 mgL^{-1} BAP, resulted in 1.98 cm long shoots as compared with 1.18 cm shoots in the MS medium supplemented with 5 mgL^{-1} Kinetin. Buah *et al.* (2010) achieved 39.5 cm in MS medium supplemented with 4.5 mg L^{-1} BAP where MS medium supplemented with the same concentration of Kinetin resulted in 25.0 cm shoots. The results on shoot length disagree with the finding of Wong (1986) who reported that Kinetin induced longer shoot than BAP. Variation in the activity of different cytokinins can be explained by their different uptake rate (Blakesley, 1991), varied translocation rates to meristematic regions and metabolic processes, in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Kaminek (1992).

Rooting can be stimulated when individual shoots are transferred to basal medium alone. However, auxins may induce further root initiation found that NAA (1 μM) was more effective than IAA. The optimum IBA concentration was found to be 1 μM. Gubbuk and Pekmezci (2004) observed that when active charcoal was added to MS medium, it was not necessary to include IBA or NAA for rooting. During our experiment there was no significant difference among all treatments in terms of rooting percentage as all the microshoots transferred into the media resulted in per cent rooting. The number and length of the roots increased gradually in media with or without auxins as the experiment progressed. At the end of three weeks

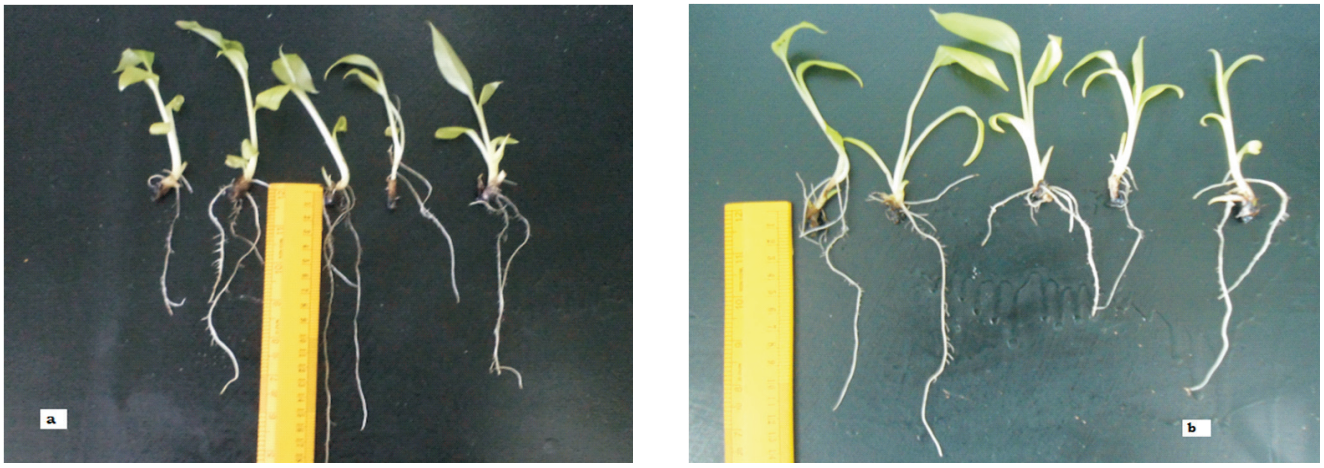

Plate 2: Number and length of primary roots as affected by kind and concentration of auxins. a) Roots induced by 1.5 mg L^{-1} IBA, b) Roots induced by 1.5 mg L^{-1} NAA

period (21 days), there were significant variations in the number of roots (Table 2) induced by different treatments. The highest number of roots per plantlet (4.90) was obtained in MS medium + 1.5 mg L^{-1} IBA + 2.0 g L^{-1} activated charcoal. This was found to be statistically on par with MS medium + 1.0 mg L^{-1} IBA + 2.0 g L^{-1} activated charcoal + 1.0 mg L^{-1} IBA which resulted in (4.70) roots. The lowest number of primary roots (2.80) was recorded with MS medium + 2.0 g L^{-1} activated charcoal + 1.0 mg L^{-1}NAA.The present finding is in conformity with that of (Rahman *et al,* 2005) and Gubbuk and Pekmezci (2004) who reported that IBA induced more number of roots than NAA. Cronauer and Krikorian and Jalil *et al.* (2003) used activated charcoal alone to induce rooting. However this result disagrees with the findings of Rahman *et al.* (2002) reported that NAA was more effective than IBA for root induction.

A significant variation in root length was also noticed among different treatments (Table-2). After 3 weeks, the highest root length (8.990 cm) was induced by the media containing MS medium + 2.0 g L^{-1} activated charcoal + 1.5 mg L^{-1} IBA while the shortest roots (4.981 cm) were observed with MS medium + 2.0 g L^{-1} activated charcoal + 0.5 mg L^{-1} NAA). The rate of increase in the root length was rapid in MS medium supplemented 2.0 g L^{-1} activated charcoal + 1.5 mg L^{-1} IBA whereas the slowest was observed with MS medium + 2.0 g L^{-1} activated charcoal + 0.5 mg L^{-1} NAA. This result agrees with the findings of Rahman *et al.* (2002, 2005,) and Gubbuk and Pekmezci (2004) who reported that IBA induced longer roots than NAA. Robert *et al.* (2013) revealed that the highest multiple shoot induction was found in MS + 5 mg/l BAP at 2.17 shoots while MS + 1 mg/l NAA + 0.2 mg/l BAP gave the longest regenerated shoots after 45 days of incubation. Highest number of roots was found in MS + 2 mg/l NAA. Therefore, the present study revealed that IBA was superior to NAA in terms of root formation for red banana and that MS medium supplemented with lower concentrations of activated charcoal (2g L^{-1}) and IBA (1.5 mg L^{-1}) was most suitable for root induction in red banana cultivar. Anbazhagan *et al.* (2014) observed half strength MS medium supplemented with IBA at concentration 1.0mg/L. was good for root formation of *in vitro* developed shoots.

In conclusion, BAP was more effective in producing more and better quality shoots than Kinetin in *in vitro* propagation of Red banana. MS medium supplemented with 1mg L^{-1} IAA and 4 mg L^{-1} BAP produced (8.80) quality shoots while the same medium supplemented with 4 mg L^{-1} Kinetin could only produce 5.60 shoots. At rooting stage, IBA induced more and longer roots than NAA. Overall more and quality shoots were produced on MS medium + 1mg L^{-1} IAA + 4 mg L^{-1} BAP while rooting was best on MS + 1.5 mg L^{-1} IBA + 2 g L^{-1} activated charcoal.

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