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Effect of Various Levels of Cytokinin and Auxin for *In-Vitro* Regeneration of Banana Cultivars

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***Abstract* – An experiment was carried out to find out the effect of various levels of cytokinin and auxin for rooting of regeneration of banana through *in vitro* culture. Excised shoot tip of banana cv. Grande Naine and Jahaji were cultured on MS medium supplemented with cytokinin (BAP) and auxins (NAA and IBA). 4.5 mg/l was found as the best concentration of BAP in induction of numerous buds, among the various concentration tested (0-6.5 mg/l BAP). The treatment resulted in the highest number of buds (an average of 7.05 and 7.2) with highest mean length of 0.65 cm and 0.7 cm of shoots were recorded in 4.5 mg/l BAP concentration in Jahaji and in Grande Naine respectively. Shoot elongation resulted best in lower concentration of BAP (1.5 mg/l). For rooting, NAA and IBA were used individually in concentrations of 0, 0.1.0.2.1 mg/l. Cent percent rooting was achieved in medium with 1 mg/l NAA. This treatment also recorded highest number of functional roots (6.33 and 5.2) in Grande Naine and Jahaji respectively with moderate root length of (2-4)cm. it was recorded that ex-vitro survival of plantlets is 98% when they were subjected to secondary hardening in media mixture of pre sterilized topsoil: FYM: sand: vermicompost: cocopeat in ratio of 1:1:1/2:1/2:1 (V/V)filled in black polybags under 75% shade net condition.**

***Keywords* – Grande Naine (AAA) & Jahaji (AAA), Shoot Tip, Growth Regulators, *In Vitro* Propagation.**

I. INTRODUCTION

Banana (*Musa spp.*) belonging to the family Musaceae, is the fourth largest important global food commodity after rice, wheat and milk in terms of gross value of production (INIBAP, 1997) North east region of India is one of the centers of both the cultivated and wild forms of banana and is known to possesses a wide genetic diversity of banana germplasm (Agharkar and Govindswami, 1951; Hore *et.al*., 1992). All the cultivated bananas are triploid seedless or seed sterile hence clonally propagated through corms and suckers. Various workers have successfully demonstrated micropropagation of banana (Vuylskeke and De Langhe, 1985; Ganapathi *et. al*., 1995).which is an excellent alternative to conventional method, with the potential to provide genetically uniform, pest and disease free planting material obtained throughout the year irrespective of season, in limited period of time and space (Khanam *et. al*., 1996 ; Razdan, 1993). For in-*vitro* regeneration of crop plants, plant growth regulators are the essential part in any artificial medium. By changing the amounts and types of growth regulators in the medium, the cells can be stimulated to develop into shoots and/ or roots or even may die, if the medium solidification is changed, the micro-propagation efficiency also altered. (Escalona *et.al*., 1999; Alvard *et.al*., 1993). In the present investigation, the objective was to study the effects of various levels of cytokinin on shoot proliferation and auxins on rooting of regenerated shoots through *in vitro* micropropagation of *Musa* cv. Grande Naine (AAA) and Jahaji (AAA).

II. MATERIALS AND METHODS

The present investigation was carried out at the Tissue Culture Laboratory, Department of Horticulture, NU: SASRD, Medziphema Campus during the year 2009-2010.shoot tips were collected from Healthy sword suckers (1-2 months old) of two *banana* cv. Grande Naine (AAA) and Jahaji (AAA), and rinsed thoroughly under running tap water for at least two hours. Roots and outer layers of tissues of the suckers were removed and washed with distilled water. The remaining portion by diluted geepol and then rinsed in running tape water for one hour Shoots tips measuring about 8 cm in length were isolated and kept in a solution of distilled water for about 1 hour to prevent blackening due to oxidation of phenolics compound. it was further treated with Bavistin @ 1 g/l for 1 hour with constant shaking in magnetic shaker and thereafter rinsed thoroughly in running tap water. Thereafter shoot tips were reduced to 5-6 cm size with a sterilized blade, and wash 2 - 3 times with distilled water and taken to inoculation room. The shoot tips were surface sterilized under aseptic condition with alcohol (70% Isopropyl alcohol) for 5 minutes with constant shaking, then left exposed for 1-2 minutes until the alcohol evaporates, followed by 2 - 3 time rinsing with distilled water. Further sterilized with 40% Sodium hypochlorite (CG) solution for 20 minutes with constant shaking and rinsed thoroughly 2-3 times with distilled water. Surface sterilant solution was prepared fresh every time. MS modified medium was used supplemented with 30 g/l sucrose, solidified with 8 g/l agar and adjusted to ph 5.8 prior to autoclaving at 121^{o} and 1.2 kg/cm^{2} pressure for 20 minutes. Excised shoot tips of 2cm high were inoculated into the medium supplemented with BAP at different concentrations of 0, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mg/l, to investigate their effects on shoot proliferation. The inoculated culture materials were kept in culture room maintained incubation temperature at 25±2^{o}c and 50-70% RH, and photoperiod of 12 hrs under florescent light (intensity of 1000 lux) and dark cycle. Each treatment was replicated three times with three explants per replication. Three sub culturing were followed at 4 weeks interval.

Media having lower concentration of BAP(0-3.5 mg/l) were used for shoot regeneration in order to induce

rooting, actively growing shoots with maximum leaves were inoculated in MS medium supplemented with NAA or IBA at concentrations of 0,0.1,0.2,1mg/l and incubated at 25±2°c temperature and 16 hr light photoperiod container grown plantlets were slowly shifted to media with sufficient height (4-5)cm, in pre sterilized coco peat filled thermo cups for acclimatization which was covered with perforated polythene bags with five holes. Ten more holes were increased in poly bags on per week basis up to two weeks. Finally the same plantlets were transferred in 75% shade net hour for secondary hardening which were planted in black poly bags filled with mixture of pre sterilized top soil:sand:FYM:vermicompost:cocopeat at the rate of 1:1:1/2:1/2:1(V/V). The experimental design was completely randomized (CRD) and the effects of treatments were tested by Analysis of Variance; differences among the treatment means were tested by Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

Apices of the two cultivars turned green after 3 to 7 days in most of the cultures inoculated which is in agreement with findings of Ranjan *et. al.,* (2001). Excessive swelling was observed at the base of the explants after 2 to 4 weeks of culture. Eight concentrations of BAP were tested in which higher level of cytokinine concentration gave shoot proliferation. Lower concentrations of BAP (0-2.5 mg/l) did not favour multiplication.

MS medium supplemented with 4.5 mg BAP/l induced earlier multiple buds initiation of Grande Naine shoot tip within 49.1 days with higher multiple buds (7.2cm) after six weeks of culture (TABLE 1).these results are in close conformity with the observations of Minas(2002) and Lima and Moraes(2006a). Repeated sub culturing of these buds at four weeks interval onto fresh medium resulted in higher bud proliferation (8.6 buds per explants) after third subcultures with moderate length of multiple buds (1.5 cm) (TABLE2, PLATE 1).

Shoot tip induction and proliferation of Jahaji (AAA), was found statistically significant in MS medium, supplemented with 4.5 mg /l BAP with highest number of multiple bud (7.05) and least shoot length (0.653 cm). However, the days to multiple buds initiation was earlier in MS medium with 3.5 mg/l BAP (45.5 days) as compared to 4.5 mg/l BAP (47.33). Similar results of maximum shoot regeneration from MS medium supplemented eith 4.0 mg/l BAP were reported by Rahman *et. al.* (2004), Lima and Moraes (2006) and Aish Muhammed *et. al.* (2007). However, in contradiction results that 5.0 mg BAP/l gave better results. (Quisen *et. al*. (2004), Rahman *et. al.* (2005) and khaldun *et. al*. (2007). In this study it was observed that rate of multiplication was different among the medium but multiplication rate was more or less same, maybe due to the explants of the same genotypes. Moreover, significant increase in the number of multiple buds (8.44) and maximum shoot length (1.19 cm) was observed in the third subculture.(TABLE 2, PLATE 2)

The multiple buds obtained from both the cultivars were transferred onto four different regeneration medium containing lower concentration of BAP. The medium containing 1.5 mg/l BAP recorded 100% response in both the cultivars (TABLE 3). In Grande Naine (AAA) maximum shoot length (6.05cm) and number of leaves (3.55) and in Jahaji (AAA), shoot length of 5.0cm and 3.99 number of leaves were recorded.. The results of both the explants in respect to regeneration were recorded significant with less variation, maybe due to physiological response of rhizomes of different explants of same genotypes.

This results, that for successful shoot elongation low level of cytokinin medium was required which is at par with various findings (Lane (1979), Jarret *et. al.* (1985), Sebastian *et. al.* (2004).

The shoots with sufficient height were transferred individually to eight modified MS medium with different concentrations of NAA or IBA. No response to rooting was recorded in absence of growth regulations and in combinations @ 1 mg/l. lower concentration of IBA induced earlier root growth but lesser functional roots as compared to NAA. Length of root was not increased with the increasing of NAA and IBA concentrations. The medium supplemented with 1 mg/l NAA was found as the best root inducing medium in this study (TABLE 4, PLATE 3). After 30 days of *in-vitro* culture, the initial roots changed in colour and new root initiation stopped except secondary and tertiary root initiation. Rahman *et.al*. (2002) reported that the number of shoots responded to rooting and their survivability was higer with NAA than IBA. These findings are in close conformity with several reports.(Bekheet and Saker, 1999; Rahman *et.al*.,2004).

For the primary hardening, only pre-sterilized coco peat was used which gave survivability percentage of 85% and 90% in Grande Naine and Jahaji respectively. Soil mixture media composing of pre-sterilized top soil: FYM: sand: vermicompost: coco peat at the rate of 1:1:½:½:1 v/v. in black poly bags gave maximum survivability of 98% ofeach for both cultivars during secondary hardening (TABLE 5).

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Table 1: Shoot multiplication of Musa spp. after 6 weeks of culture

BAP Concentration mg/l	Cultivars					
	cv. Grand Naine			cv. Jahaji		
	Days to multiple bud initiation	No of multiple buds	Length of buds(cm)	Days to multiple bud initiation	No of multiple buds	Length of buds(cm)
0	-	-	-	-	-	-
1.5	-	-	-	-	-	-
2.5	-	-	-	-	-	-
3.5	54.3c	5.2b	0.59b	45.5d	5.16b	0.71b
4.5	49.1d	7.2a	0.70b	47.33c	7.05a	0.65b
5.5	58.2b	5.8b	0.94a	60.16b	5.66b	0.97a
6.5	62.6a	3.1c	1.1a	62.5a	1.66c	1.03a
SEm±	1.4	0.23	0.08	0.527	0.350	0.080
$CD_{0.05}$	3.01	0.51	0.17	1.113	0.75	0.17

Values within parentheses indicate percentage of response
Means within columns separated by Duncan's Multiple Range Test, P = 0.05
Means followed by the same letter shown in subscript (s) are not significant different

Table 2: Regeneration of Musa cultivar at different sub culturing cycles in MS medium found best for both cultivars

Subculture	Grand Naine		Jahaji	
	No. of multiple bud explants	Length(cm)	No. of multiple bud explants	Length of multiple buds(cm)
1^{st}	4.66c	1.3a	4.66c	0.78b
2^{nd}	6.5b	1.7a	6.77b	1.06a
3^{rd}	8.6a	1.5a	8.44a	1.19a
SEm±	0.23	0.23	0.60	0.09
$CD_{0.05}$	0.53	0.56	1.47	0.23

Values within parentheses indicate percentage of response
Means within columns separated by Duncan's Multiple Range Test, P = 0.05
Means followed by the same letter shown in subscript (s) are not significantly different

Table 3: Regeneration rate of *Musa* cultivar in different modified MS medium

BAP concentration (mg/l)	Cultivars			
	Grand Naine		Jahaji	
	Length of shoot (cm)	No of leaves per plant	Length of shoot (cm)	No of leaves per shoot
0	5.02b	2.11b	4.1bc	2.6b
1.5	6.05a	3.55a	5.0a	3.9a
2.5	5.27b	2.88b	4.2ab	3.11b

3.5	4.05c	1.5c	3.23c	1.9c
SEm±	0.16	0.23	0.09	0.10
$CD_{0.05}$	0.39	0.53	0.22	0.23

Values within parentheses indicate percentage of response
Means within columns separated by Duncan's Multiple Range Test, P = 0.05
Means followed by the same letter shown in subscript (s) are not significantly different

Table 4: Root multiplication rate of banana cultivar in different modified MS Media

Medium	Grand Naine		Jahaji	
	No. of functional root/explants	Length of root (cm)	No. of functional root/explants	Length of root (cm)
MS+0	-	-	-	-
MS +1mg/l NAA	6.33_{a}	2.07_{ab}	5.2_{a}	4.99_{b}
MS +1mg/l NAA+1mg/l IBA	-	-	-	-
MS +1mg/l IBA	5.77_{a}	3.11_{a}	4.9_{ab}	5.62_{a}
MS +0.1mg/l NAA	3_{bc}	1.10_{bc}	-	-
MS +0.2mg/l NAA	5.33_{ab}	1.98_{ab}	4.4_{b}	2.55_{cd}
MS +0.1mg/l IBA	2.33_{cd}	1.33_{b}	2.33_{c}	2.05_{d}
MS +.2mg/l IBA	2.5_{cd}	1.41_{b}	2.55_{c}	3.36_{c}
SEm±	1.47	1.47		1.44
$CD_{0.05}$	3.12	3.12		3.07

Values within parentheses indicate percentage of response
Means within columns separated by Duncan's Multiple Range Test, P = 0.05
Means followed by the same letter shown in subscript (s) are not significantly different

Table: 5 Survivability percentage of plantlets during primary and secondary hardening of *Musa cv.* Grand Naine (AAA) and Jahaji (AAA)

Plantlets taken	Survivability (%) of Grand Naine (AAA)		Survivability (%) of Jahaji (AAA)	
	Primary harden	Secondary harden	Primary harden	Secondary harden
30	85	98	90	98