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Optimization of *in vitro* multiplication for exotic banana (*Musa* spp.) in Pakistan

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The present attempt aimed to optimize micropropagation protocols supplemented with different concentrations and combinations of benzylaminopurine (BAP) (0, 2, 4, 6 mg L^{-1}) and indole acetic acid (IAA) (0.5 and 1.0 mg L^{-1}). Exotic banana (*Musa* spp) genotypes GCTCV-215 (AAA), 'Yangambi' Yangambi Km-5 (AAA) and FHIA-23 (AAAA) were used in research work. Experiments were conducted at Plant Tissue Culture Laboratory of Nuclear Institute of Agriculture (NIA), Tando Jam. Data collected for *in vitro* shoot consists of the following parameters: days for bud initiation, rate of shoot proliferation (%), number of multiple shoots, shoot length (cm) and fresh mass of shoot (g). Significant (p≤ 0.05) variations were observed for varieties, treatments and varieties x treatment for all the parameters. Synergistic effects of BAP and IAA were observed in GCTCV-215 and Yangambi Km-5. Out of various treatments, best concentration for multiple shoot in short period of time for GCTCV-215 and Yangambi Km-5 was found in 4.0 mg /l BAP + 0.5 mg L^{-1} IAA. Maximum fresh mass of shoot observed at same concentration and combination of BAP and IAA and for shoot length combination of 4.0 mg L^{-1} BAP with 1.0 mg L^{-1} IAA was found to be most suitable for GCTCV-215 and Yangambi Km-5. FHIA-23, show better performance in MS medium supplemented with only BAP at concentration 4.0 mg/L^{-1}. After development of root, *in vitro* plantlets were shifted from growth room to green house in polythene bags containing garden soil and humus mixture in ratio (1:1).

Key words: Micropropagation efficiency, exotic *musa* genotype, growth regulators.

INTRODUCTION

Bananas are large perennial herb (*Musa* spp.) belonging to the monocotyledonous family Musaceae. Banana is an important and widely grown fruit crop in the tropical and subtropical regions of the world (Darvari et al., 2010; Rahman et al., 2013). Banana serves as a source of instant energy and has lots of health benefits. In Pakistan, during the last five years banana has been grown on approximately 30 m ha with annual average production of 137 thousand tons (FAOSTAT, 2014). Due to popularity of the banana fruits for their nutritional properties the demand is increasing continuously and hence production of healthy planting material is necessary (Al-Amin et al., 2009). But because of the cultivation of susceptible cultivars, low soil fertility, higher wind velocity, drought stress and plants diseases, the banana production in Pakistan has reduced enormously. Genetic improvement

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in banana through conventionally approaches is restricted mainly due to reasons like variable polyploidy level, seedlessness, low female fertility and rising of asexual progeny with desirable characters (Bidabadi et al., 2012; Devendrakumar et al., 2013). The long term option for increasing banana yield could be the use of resistant varieties. Their multiplication through *in vitro* means could help to reduce the spread of pathogen to newer areas (Arvanitoyannis et al., 2008; Babita et al., 2013; Waman et al., 2014).

Success in *in vitro* multiplication is based on the growth and differentiation of plant tissues, which is viable only by the addition of suitable growth regulators (Gaspar et al., 2003). In shoot tip culture, cytokinins are known to enhance buds growth and shoot formation, while auxins promote root induction and development (North et al., 2012; Ngomuo et al., 2014). Benzylaminopurine (BAP) combined with auxins (indole acetic acid and naphthalene acetic acid) exhibit synergistic effect and hence has also been used by number of researchers (Al-Amin et al., 2009; Jafari et al., 2011; Sipen and Davey, 2012; Ngomuo et al., 2013). Tissue culture induced genetic variation in clonally propagated plant populations which may be genetic or epigenetic in nature; these variations are called 'somaclonal variation' (Larkin and Scowcroft, 1981; 1983). Such variation commonly occur in both *in vitro* and *in vivo* propagated *Musa* and had both positive and negative impact. Sometimes these variations create major obstacle for clonal uniformity (Nwauzoma and Jaja, 2013) but also exploited as source of genetic improvement of vegetatively propagated crops.

The present studies aimed at optimizing the kind and concentration of growth regulators for obtaining improved multiplications rate in high yielding genotypes of banana. This could help for area expansion of these superior exotic types in our country.

MATERIALS AND METHODS

Explants

For the establishment of *in vitro* shoot tip culture, suckers of three exotic genotypes of banana (*Musa* spp.) were selected from experimental field of Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. Of these two are triploid *viz*. 'Yangambi' Yangambi Km-5 (AAA) and Giant Cavendish Tissue Culture Variant GCTCV-215 (AAA), while Fundación Hondureña de Investigaciones Agrícolas FHIA-23 (AAAA) is a tetraploid.

Surface sterilization of explants

For decontamination, isolated explants from suckers were treated with 70% alcohol and 10% sodium hypochlorite separately for one and 20 min, respectively. After washing with sterile distilled water explants was trimmed to a size of about 6 to 8 mm.

Media and culture condition

Prepared explants were then cultured onto MS (Murashige and Skoog, 1962) basal medium with or without supplemental growth regulators. Culture medium for shoot induction and multiplication was prepared by supplemented with the MS medium with nine combinations of BAP with or without IAA (Table 1). The pH of medium was adjusted to 5.8 prior to placing in microwave oven. Prepared media were then poured into sterilized jars and autoclaved for 20 min at 121°C. For the establishment of culture all *in vitro* culture jars were transferred to growth room, at 25 ± 2°C temperature under 16/ 8 h light period provided by cool white florescent tubes with light intensity 2000 lux (27 µM m^{-2} s^{-1}).

Table 1. Different concentrations of BAP (with or without IAA) used during study.

Treatments	Concentrations (mg L^{-1})
MS Basal	0
BAP	2.0
BAP	4.0
BAP	6.0
BAP+IAA	2.0+0.5
BAP+IAA	2.0+1.0
BAP+IAA	4.0+0.5
BAP+IAA	4.0+1.0
BAP+IAA	6.0+0.5
BAP+IAA	6.0+1.0

Root induction media

For rooting, *in vitro* healthy shoots (of 4 to 5 cm) were transferred to half strength MS medium supplemented with 1.0 mg L^{-1} indole butyric acid (IBA).

Data collection and statistical analysis

Experiment was conducted in Completely Randomized Design (CRD) with four replications per treatment. Data were taken at 4 weeks intervals after subculture and was recorded every day for bud initiation per explant, proliferation rate (%), multiple shoots per explant, fresh mass (g) and shoots length (cm). Data statistically analyzed, was based on mean values per treatments and using analysis of variance (ANOVA). Statistical software STATISTIX (8.1version) was used.

RESULTS AND DISCUSSION

In the present work, effects of different BAP concentrations (2.0, 4.0 and 6.0 mg L^{-1}) with or without IAA (0.5 and 1.0 mg L^{-1}) were studies for optimizing the protocol for effective multiple shoot of the exotic *Musa* genotypes. The results are presented in Tables 2 to 4 and their analysis of variance is presented in Table 5. Result show significant ($p \leq 0.05$) differences for all studied parameters.

Effects of BAP and IAA on shoot proliferation and multiplication

Results indicate that in GCTCV-215 and Yangambi Km-5

Table 2. Effect of different concentrations of BAP and IAA on explant of GCTCV-215 in MS medium.

Treatments BAP+IAA mg L^{-1}	Explant/ Treatment	Variables				
		Rate of proliferation (%)	Average no of multiple shoot per explant	Days for bud initiation	Average fresh mass (g)	Average shoot length (cm)
0.0 + 0.0	10	13	1.25^{m-o}	17.0^{j}	2.12^{n-p}	2.00^{n}
2.0 + 0.0	10	25	2.50^{jk}	16.2^{jk}	2.80^{i-l}	2.56^{lm}
4.0 + 0.0	10	28	2.75^{ij}	14.0^{l}	3.00^{i-k}	3.25^{ij}
6.0 + 0.0	10	18	1.75^{lm}	14.5^{l}	2.75^{j-l}	3.83^{gh}
2.0 + 0.5	10	65	6.50^{c}	13.0^{m}	6.08^{c}	4.20^{ef}
2.0 + 1.0	10	75	7.50^{b}	11.2^{n}	7.32^{b}	4.99^{d}
4.0 + 0.5	10	88	8.75^{a}	7.0^{q}	8.77^{a}	5.16^{cd}
4.0 + 1.0	10	55	5.50^{d}	12.0^{n}	5.26^{e}	6.80^{a}
6.0 + 0.5	10	20	2.00^{kl}	18.0^{i}	3.63^{h}	3.18^{i-k}
6.0 + 1.0	10	18	1.75^{lm}	19.5^{gh}	3.18^{i}	2.05^{n}

Mean followed by dissimilar letters in a column are significantly different by least significant difference (LSD) test at P≤ 0.05.

Table 3. Effect of different concentrations of BAP and IAA on explant of Yangambi Km-5 in MS medium.

Treatments BAP+IAA mg L^{-1}	Explant/ Treatment	Variables				
		Rate of proliferation (%)	Average no of multiple shoots per explant	Days for bud initiation	Average fresh mass (g)	Average shoot length (cm)
0.0 + 0.0	10	10	1.00^{n-p}	28.0^{b}	2.25^{m-o}	1.65^{o}
2.0 + 0.0	10	20	2.00^{kl}	24.2^{d}	2.34^{m-o}	2.26^{mn}
4.0 + 0.0	10	25	2.50^{jk}	18.0^{i}	2.56^{lm}	3.06^{jk}
6.0 + 0.0	10	13	1.25^{m-o}	16.2^{jk}	2.00^{op}	2.87^{kl}
2.0 + 0.5	10	38	3.75^{fg}	15.5^{k}	4.02^{gh}	3.42^{i}
2.0 + 1.0	10	48	4.75^{e}	13.7^{lm}	4.75^{f}	4.00^{fg}
4.0 + 0.5	10	65	6.50^{c}	8.0^{p}	5.82^{cd}	5.50^{c}
4.0 + 1.0	10	35	3.50^{f-h}	$19.^{2gh}$	3.79^{gh}	6.11^{b}
6.0 + 0.5	10	18	1.75^{lm}	20.0^{g}	2.50^{l-n}	2.94^{jk}
6.0 + 1.0	10	10	1.00^{n-p}	22.7^{e}	3.06^{ij}	2.00^{n}

Mean followed by dissimilar letters in a column are significantly different by least significant difference (LSD) test at P≤ 0.05.

combination of BAP and IAA significantly (p≤ 0.05) induced bud proliferation and multiplication in short period of days (Table 2 and 4). However, in FHIA-23 BAP alone had significant effect on bud proliferation as well as on multiplication (Table 4). As compared to control, in GCTCV-215

Table 4. Effect of different concentrations of BAP and IAA on explant of FHIA-23 in MS medium.

Treatments BAP+IAA mg L-1	Explant/ Treatment	Variables				
		Rate of proliferation (%)	Average no of multiple shoot per explant	Days for bud Initiation	Average fresh mass (g)	Average shoot length (cm)
0.0 + 0.0	10	8	0.75^{op}	29.7^{a}	2.47^{l-n}	0.80^{p}
2.0 + 0.0	10	15	1.50^{l-n}	26.2^{c}	2.85^{i-l}	2.08^{n}
4.0 + 0.0	10	48	4.75^{e}	25.0^{d}	5.60^{de}	4.96^{d}
6.0 + 0.0	10	10	1.00^{n-p}	21.0^{f}	2.61^{k-m}	2.54^{lm}
2.0 + 0.5	10	33	3.25^{g-i}	19.0^{h}	4.08^{g}	3.00^{jk}
2.0 + 1.0	10	40	4.00^{f}	10.0^{o}	3.76^{gh}	4.40^{e}
4.0 + 0.5	10	30	3.00^{h-j}	16.3^{jk}	3.07^{ij}	4.01^{fg}
4.0 + 1.0	10	25	2.50^{jk}	22.7^{e}	2.81^{i-l}	3.49^{hi}
6.0 + 0.5	10	10	1.00^{n-p}	25.0^{d}	2.56^{lm}	2.16^{n}
6.0 + 1.0	10	5	0.5^{0p}	27.5^{b}	1.75^{p}	1.63^{o}

Mean followed by dissimilar letters in a column are significantly different by least significant difference (LSD) test at P≤ 0.05.

Table 5. Analysis of Variance (ANOVA) for days to bud initiation, number of shoots, fresh mass (g) and shoot length (cm)

Source of variation	Mean sum of squares of shoot parameters				
	DF	Days to bud initiation	Number of shoots	Fresh mass (g)	shoot length (cm)
Replications	3	0.343	0.7667	0.0608	0.0940
Varieties	2	642.187*	33.8083*	21.4006*	7.9918*
Treatments	9	273.056*	42.4778*	19.6365*	20.5552*
V x Treat	18	24.702*	5.0583*	5.8150*	2.0401*
Error	87	0.335	0.2149	0.0800	0.0612
Total	119	--	--	--	--

The results are for the mean of 4 replicate (Significant at P≤0.05 =*).

(8.75 shoots/ explant) and Yangambi Km-5 (6.50 shoots/ explant) the highest numbers of multiple shoots with high proliferation rate (87 and 65%), respectively, were observed in MS media concentrated with 4.0 mg L^{-1} BAP + 0.5 mg L^{-1} IAA while in FHIA-23, highest number of shoot multiplication (4.75 shoots/ explant) with high shoot proliferation rate (48%) was observed in medium supplemented with 4.0 mg L^{-1} BAP (Figure 1). Results indicate that media containing with high level of BAP alone or in combinations with IAA relatively decreased the number of shoot multiplication in all genotypes. Low concentration also showed slight increase in bud proliferation when compared with control (Figure 3). Concentration of BAP 4.0 mg L^{-1} along with IAA was found optimum for selected varieties of *musa.* The results agree with the findings of Muhammad et al. (2007). They found superior multiplication ratio at same concentration of 4.0 mg L^{-1} BAP along with 1.0 mg L^{-1} IAA. Habiba et al. (2002)

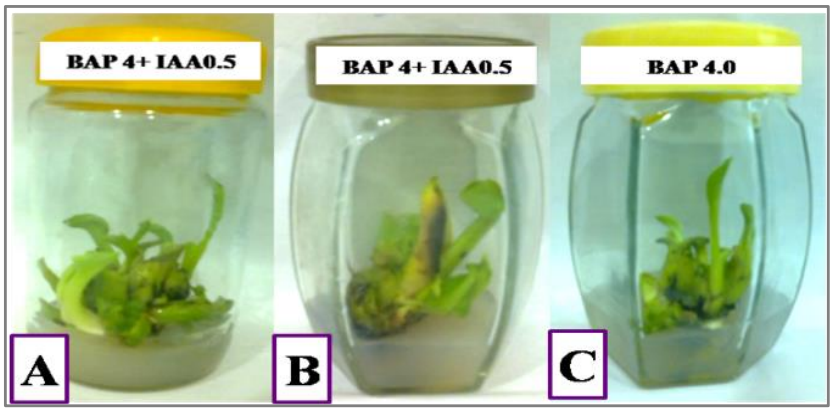

Figure 1. Multiple shoot produced by (A) GCTCV-215, (B) Yangambi Km-5 and (C) FHIA-23.

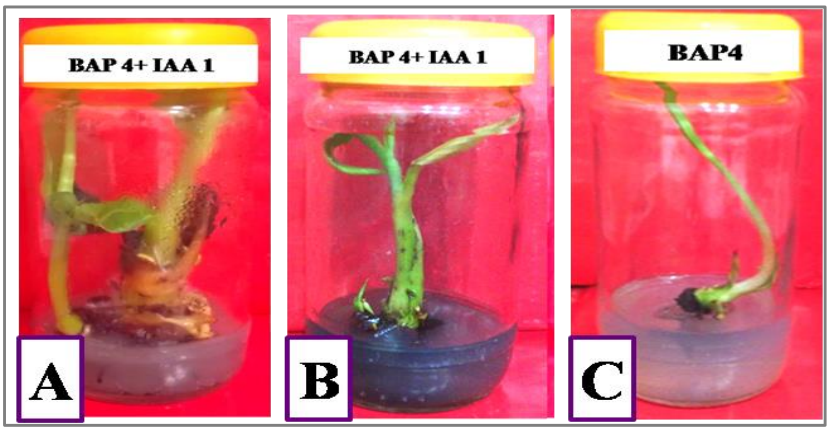

Figure 2. Variation in shoot length (A) GCTCV-215, (B) Yangambi Km-5 and (C) FHIA-23.

and Ahmed et al. (2014), also reported synergistic effect of BAP and IAA at nearly similar combination of 4.0 mg L^{-1} BAP and 2.0 mg L^{-1} IAA. Frequency of multiple shoot formation was 3 to 4 folds due to sub culture in the same fresh media. Current findings supported the earlier studies, which suggest that rate of shoot multiplication was dependent on specific genotype. Gubbuk and Pekmezcu (2004) and Ngomuo et al. (2013) suggested that apart from the genotypes behavior, shoot proliferation was also affected by exogenous cytokinin concentration in growth medium. Suitable cytokinins concentrations in medium inhabit apical dominancy and support initiation of lateral shoots (Jafari et al., 2011). Besides, the presence of exogenous phytohormones, *in vitro* organogenesis depends on the interaction of various factors associated with the endogenous phytohormones, their concentrations, and rate of metabolisms and presence of nutrients in medium (Skoog and Miller, 1957; Ammirato, 1986; Ahmed et al., 2014).

Effects of BAP and IAA on fresh mass and shoot length

Result shows that fresh mass significantly ($p \leq 0.05$) increased at 4.0 mg L^{-1} concentration of BAP along with 0.5 mg L^{-1} IAA in GCTCV-215 and Yangambi Km-5 (Table 3 and 4). Among nine treatments, as compared to control GCTCV-215 (8.77 g) ,Yangambi Km-5 (5.82 g) showed maximum fresh mass in MS medium supplemented with 4.0 mg L^{-1} BAP + 0.5 mg L^{-1} IAA. Whereas, in FHIA-23 (5.60 g), MS medium supplemented with 4.0 mg L^{-1} BAP alone gave good response (Figure 2). It was evaluated that hormonal response is specific genotypic dependent. In various treatments MS media supplemented with low concentration of BAP 2.0 and 4.0 mg/ L^{-1} along with IAA, was considered as optimal for production of maximum fresh mass for studied *Musa* genotypes. Quite similar synergistic effect of BAP and IAA on maximum regeneration of shoot at concentration 2.0 and 0.5 mg L^{-1},

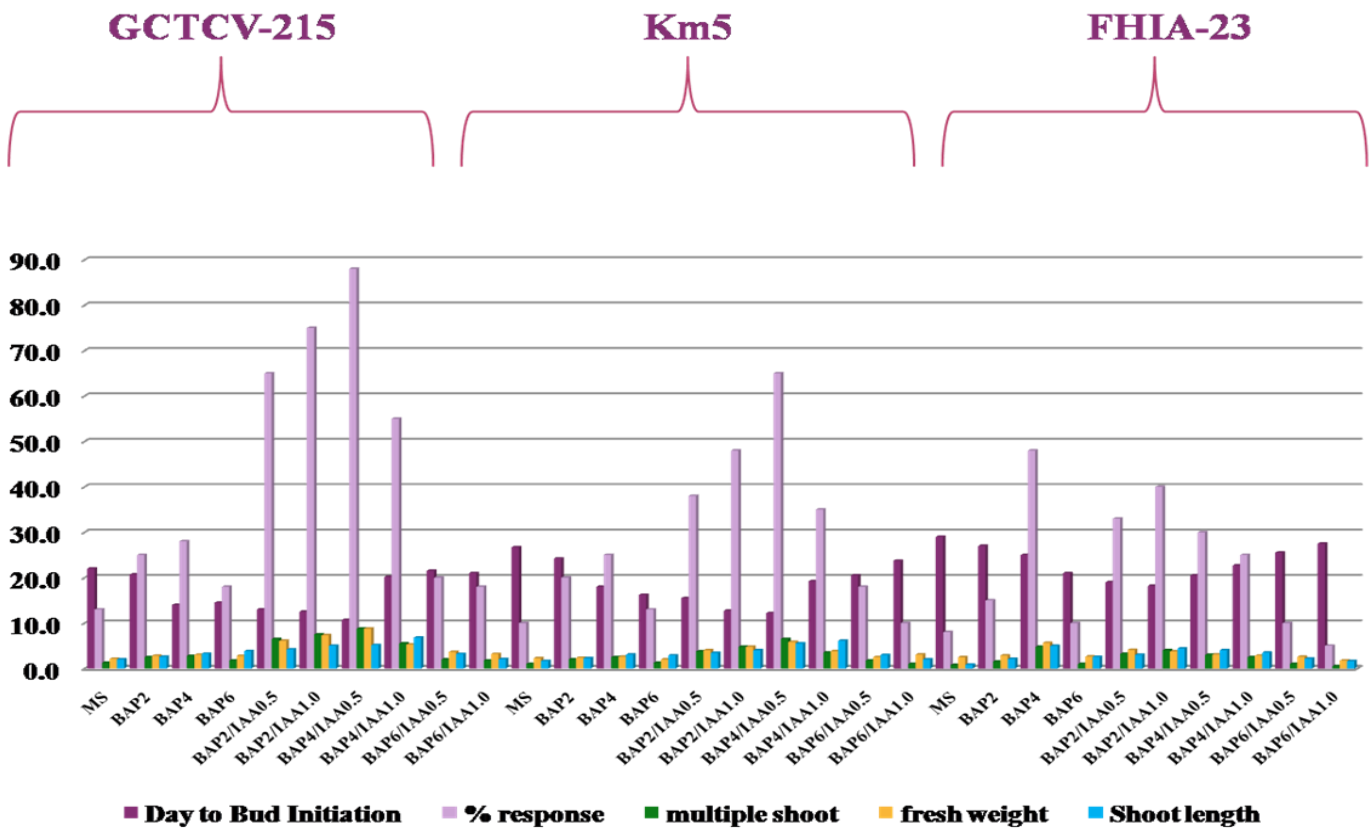

Figure 3. Effect of BAP and IAA on *in vitro* shoot tip culture of all genotypes.

respectively, was reported by Anbazhagan et al. (2014). In GCTCV-215 (6.80 cm) and 'Yangambi' Km-5 (6.11 cm), highest shoot length as compared to control was observed in medium concentrated with 4.0 mg L^{-1} BAP + 1.0 mg L^{-1} IAA, while in FHIA-23 (4.96 cm), highest shoot length was observed in 4.0 mg L^{-1} BAP alone (Figure 3). Results indicate that media supplemented with high level of BAP alone or in combinations with IAA relatively decreased the shoot length in all genotypes. In all genotypes, cytokinin (BAP) and auxin (IAA) showed an effective synergistic effect on shoot length. Jafari et al. (2011) and Dhed et al. (1991) reported that combination of BAP with IAA become more effective for shoot elongation. Other researchers reported nearly similar effect of BAP and IAA on shoot length (Iqbal et al., 2013; Rahaman et al., 2013; Ahmed et al., 2014). According to examined data for highest shoots length, concentration of BAP 4.0 mg/L^{-1} in interaction with 1.0 mg/L^{-1} IAA was suggested as optimal for significant shoot length.

Conclusion

Presence of BAP along with IAA in the culture medium induced efficient shoot multiplication in selected genotypes than BAP alone and the effect was genotype dependent. The optimum concentration of BAP for *in vitro* shoot multiplication of GCTCV-215, Yangambi Km-5 and FHIA-23 was 4.0 mg L^{-1} with 0.5 mg L^{-1} IAA. Hence, it could be used in future for *in vitro* propagation of these varieties of banana. It was also noticed that two genotypes GCTCV-215(AAA) and Yangambi (AAA) gave significant response in most defined media as compared to FHIA-23 (AAAA). Both belong to similar genomic constitution (triploid). So, maybe all genotypes behaved according to their ploidy level.

In future further study is required to carry out research work on the influence of ploidy level on micropropagation efficiency in Banana.

Conflict of interests

The author(s) did not declare any conflict of interest.

REFERENCES

Ahmed S, Sharma A, singh AK, Wali VK, Kumari P (2014). *In vitro* multiplication Banana (*Musa* sp.)cv. Grain Naine. Afr. J. Biotechnol.13 (27):2696-2703.

Al-Amin MD, Karim MR, Amin MR, Rahman S, Mamun ANM (2009). *In vitro* micropropagation of banana *(Musa* spp.*)*. Bangladesh J. Agric. Res. 34(4):645-659.

Ammirato PV (1986). In: Evans DA, Sharp WR, Ammirato PV, Yamada Y. (Eds.). Hand book of plant cell culture, Macmillan, New York. 4: 457-499.

Anbazhagan M, Balachandran B, Arumugam K (2014). *In vitro* propagation of *Musa* spp (banana). Int. J. Curr. Microbiol. Appl. Sci. 3(7): 399-404.

Arvanitoyannis IS, Mavromatis A G, Grammatikaki-Avgeli G, Sakellariou M (2008). Banana: cultivars, biotechnological approaches and genetic transformation. Int. J. Food Sci. Technol. 43:1871-1879.

Babita, Rattanpal HS, Thakur KK (2013). Recent advances in banana improvement through biotechnological approaches. J. Chem. Biol. Phys. Sci. 3(3):1853-1862.

Bidabadi SS, Meon S, Wahab Z, Subramaniam S, Mahmood M (2012). Induced mutation for enhancing variability of banana *(Musa* spp*.)* shoot tip cultures using (EMS). Aust. J. Crop Sci. 6(3):391-401.

Darvari FM, Sariah M, Puad MP, Maziah M (2010). Micropropagation of some Malaysian banana and plantain (*Musa* sp.) cultivars using male flowers. Afr. J. Biotechnol. 9(16):2360-2366.

Devendrakumar, Anbazhagan M, Rajendran R (2013). *In vitro* propagation of banana (*Musa acuminata* L.) cv. Cavendish Dwarf. Int. J. Res. Biomed. Biotechnol. 3 (3): 44-46.

Dhed D, Dumortier F, Panis B, Vuylsteke D, De Langhe E (1991). Plant regeneration in cell suspension cultures of cooking banana 'Bluggoe' cultivar (*Musa* spp. ABB group). Fruits 46: 125-135.

FAOSTAT (2014). Food and Agricultural Organization of the United Nations Statistical Database. From http://faostat.fao.org.

Gaspar TH, Kevers C, Faivre-rampant O, Crevecoeur M, Penel CL, Greppin H, Dommes J (2003). Changing concepts in plant hormone action. *In vitro* Cell. Dev. Biol. Plant 39:85-106.

Gubbuk H, Pekmezcu M (2004). *In vitro* propagation of some new banana types (*musa* spp.). Turk. J. Agric. 28:355-361.

Habiba U, Reza S, Saha ML, Khan MR, Hadiuzzaman S (2002).Endogenous bacterial contamination during *in vitro* culture of table banana : identification and prevention. Plant Tissue Cult. 12(2):117-124.

Iqbal MM, Muhammad A, Hussain I, Bilal H (2013). Optimization of *in vitro* micropropagation protocol for banana (*Musa sapientum* L.) under different hormonal concentration and growth media. Int. J. Agric. Innov. Res. 2(1):1473-2319.

Jafari N, Othman RY, Khalid N (2011). Effect of BAP pulsing on *in vitro* shoot multiplication of *Musa acuminate* (banana) cv. berangan. Afr. J. Biotechnol. 10(13):2446-2450.

Larkin PJ, Scowcroft WR (1981). Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Larkin PJ, Scowcroft WR (1983). Somaclonal variation and crop improvement. In: Kosuge T et al (eds) Genetic engineering of plants: an agricultural perspective. Plenum. New York. pp. 289-314.

Muhammad A, Rashid H, Hussain I (2007). Proliferation-rate effects of BAP and kinetin on banana (*Musa* spp. Aaa group) 'Basrai'. Hortic. Sci. 42(5):1253-1255.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Ngomuo M, Mneney E, Ndakidemi P (2013). The effects of auxins and cytokinin on growth and development of (*Musa* sp.) var. "Yangambi" explants in tissue culture. Am. J. Plant Sci. 4:2174-2180

Ngomuo M, Mneney E, Ndakidemi PA (2014). The *in vitro* propagation techniques for producing banana using shoot tip cultures. Am. J. Plant Sci. 5:1614-1622.

North JJ, Ndakidemi PA, Laubscher CP (2012). Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of strelitzia reginae. Int. J. Phys. Sci.7 (4):638-646.

Nwauzoma AB, Jaja ET (2013). A review of somaclonal variation in plantain (*Musa* spp.) mechanisms and applications. J. App. Biosci. 67:5252 – 5260.

Rahman S, Biswas N, Hassan MM, Ahmed MG, Mamun ANK, Islam MR, Moniruzzaman M, Haque ME (2013). Micro propagation of banana (*Musa* sp.) cv. Agnishwar by *in vitro* shoot tip culture. Int. Res. J. Biotechnol. 4(4):83-88.

Sipen P, Davey MR (2012). Effects of n^6-benzylaminopurine and indole acetic acid on in vitro shoot multiplication, nodule-like meristem proliferation and plant regeneration of malaysian bananas (*musa* spp.). Trop. Life Sci. 23(2):67-80.

Skoog F, Miller CO (1957). Chemical regulation of growth and organ formation in palnt tissue cultur *invitro*. In the biological action of growth substances. (Ed) Porter. A.K. Exot. Biol. 11:118-131.

Waman AA, Bohra P, Sathyanarayana BN (2014). Not all sugars are sweet for banana multiplication. *in vitro* multiplication, rooting and acclimatization of banana as influenced by carbon sources-concentrations interactions. *In vitro* Cell Dev. Biol. Plant 50:552-560.