INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 9 | ISSUE 1 | APRIL, 2016 | 204-210

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/9.1/204-210

Mass *in-vitro* micro propagation of banana (*Musa* sp.)

■ NAMRATA KUMARI* AND PRAGATI MISRA

Department of Molecular and Cellular Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, ALLAHABAD (U.P.) INDIA

ARITCLE INFO

Received : 21.01.2016
Revised : 28.02.2016
Accepted : 08.03.2016

KEY WORDS :
In-vitro: In glass; NaOCl : sodium hypochlorite; BAP: 6-Benzyl amino purine; IAA: Indole-3-acetic acid;IBA:Indole-3-Butyric Acid

*Corresponding author:

ABSTRACT

Production of Banana is constrained by many biotic as well as abiotic factors. The production of disease free planting material of banana by meristem culture technique is an urgent need.An experiment has been designed to develop a suitable and efficient protocol for plant regeneration in banana through micro propagation.The surface sterilization of banana plant is the most difficult practice.So it is very important to optimize the concentration and duration of different sterilants for explants surface sterilization. The best sterilants for the surface sterilization of the explant of banana were 0.1 per cent $HgCl_2$ for 5 min duration followed by NaOCl (40 %, commercially available) solution for 8 min duration and 70 per cent ethyl alcohol for 2 min. And the best medium for establishment of banana explant was supplemented with 3mg/l BAP, 0.2mg/l IAA with 30 mg ascorbic acid followed by Proliferation with 5mg/l BAP,0.5mg/l IAA with 30 mg ascorbic acid and 10 mg/l BAP,0.5 mg/l IAA with 30 mg ascorbic acid.Significantly highest number of roots and greater root length were produced by 0.5 mg/l IAA + 0.5 mg/l IBA.

How to view point the article : Kumari, Namrata and Misra, Pragati (2016). Mass *in-vitro* micro propagation of banana (*Musa* sp.). *Internat. J. Plant Protec.*, **9**(1) : 204-210.

INTRODUCTION

Banana, fourth most important food commodity on earth and the second largest food-fruit crops of the world has great socio-economic significance in India (Aquil *et al*., 2012). The banana (*Musa* spp.) belonging to the family *Musaceae*. Banana is rich in carbohydrate, minerals, phosphorus, calcium, potassium and vitamin-C is popular for its year round availability, abundant production as well as high acceptability to the consumers. The largest producers are Latin America and Asia.Its production and as such export is constrained by many biotic as well as abiotic factors (Aquil *et al*., 2012). The major constraint of traditionally propagated banana is the lack of ready availability of disease free quality planting material in large quantity at any given time. Thus, the urgent need for a large amount of disease-free planting material triggered the development of the banana meristem culture technique. The technology also rejuvenates the plants resulting in more vigorous growth, higher yields, better quality fruits, earlier fruiting and more uniform crop than those produced by conventional means (Kahangi, 2010). Banana is a globally important fruit crop with 97.5 million tones of production.

A review of literature revealed that micro propagation of banana has been dealt with to a great extent.Kalimuthu *et al.* (2007) developed a complete protocol for micro propagation of *Musa sapientum* using shoot meristems to induce Multiple shoots.Sheidai *et al.* (2009) established an efficient medium culture for clonal mass propagation for the propagation of banana (*Musa acuminate* L.).Al-Amin *et al.* (2009) produced the highest number of roots by 0.5 mg/lit. IAA + 0.5 mg/lit. IBA .Gabriela (2011) revealed the fact that from cytokinine, benzyl adenine (BA) with adenine sulphate in low dose of 1 mg/lit. has stimulated a good regeneration percentage (80%), with a balanced number of plants (about 8 plants/explant), but also the formation of nodules along the root system, much thickened as in the absence of adenine sulphate.Vora and Jasrai (2012) evaluated impact of various fruit juices on *in vitro* shoot-multiplication of banana. After keeping the above in view, the main objectives of the work were to optimize the concentration and duration of different sterilants for explants sterilization in banana and develop a suitable and efficient protocol for plant regeneration in banana through clonal propagation.

MATERIAL AND METHODS

Collection of explant (sucker collection):

Healthy meristematic shoot tips from sword suckers of field grown plants were used for the establishment of the initial culture. In the laboratory, outer leaves were peeled off until the explants were 3 cm in height and 1 cm at the base.

Preparation and surface sterilization of explant:

Plant materials orfield grown sword suckers extracted from fields carry a wide range of contaminants. Different concentration of disinfectant $HgCl_2$,70 per cent ethanol and sodium hypochlorite (NaOCl) have been proven satisfactory in making plant tissue aseptic. Bavestin and Indofilis also used for sterilization.

Establishment, proliferation and subculture of *in vitro* banana explant:

The isolated and surface sterilized explants with a meristem was directly inoculated to 20 ml of MS medium which was supplemented with different concentrations of Auxins (BAP), cytokinins (IAA,Kinetin),Adenine sulphate and ascorbic acid.Kinetin and Adenine sulphate were not used for Proliferation and subsequent regeneration of *in vitro* shoots. Explants were subcultured into fresh mediumto control blackening and the blackish tissues on the explants were removed. It was repeated 10 days interval for about one month to minimize further blackening of the tissues.

Rooting and hardening:

When the shoots were grew about 3-5 cm in length with 3-6 well developed leaves, they were rescued aseptically and again cultured on freshly prepared medium containing different combinations of phytohormones (IAA, IBA) for root induction. Observation recorded on the basis of Contamination percentage, Number of shoots per explants and Mean shoot length.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Regeneration of shoot from meristem explants :

Regeneration of banana plantlets through meristem culture offers a unique scope of developing planting materials. Different concentrations of cytokinin and auxin were supplied with MS medium for the induction and proliferation of multiple shoot from shoot tip.

Establishment of suitable concentration and duration of sterilants for banana tissue culture:

In the present study,the ex-plant of banana showed best result means minimum contamination when treated with 0.1 per cent $HgCl_2$ for 5 min followed by NaOCl (40 %, commercially available) solution for 8 min. The gradual increase of the concentration and duration of both chemicals showed the remarkable decrease in contamination of the explants. Effect of various treatments for sterilization of the banana explant were observed in Table 1.

In the banana micro propagation, the surface srerilization is a very important aspect. For *in vitro* culture initiation explants are normally collected from field grown plants, so the plant material is liable to be contaminated by micro-organism. (Mendes *et al.*, 1999 and Mahammad *et al.*, 2004) stated that Sodium hypochlorite is the most commonly used disinfectant for surface

sterilization of banana explants. Some other investigators (Benerjee and Sharma, 1988 and Habiba *et al.*, 2002) have replaced sodium hypochlorite with low concentration of mercuric chloride. (Van den Houwe, 1998; Nandwan *et al.*, 2000) suggested that sometimes explants are treated with fungicides and antibiotics to minimize the contamination in *in vitro* cultures. (Silva *et al.*, 1998; Rahman *et al.*, 2004 and Jalil *et al.*, 2003) used Ethanol for disinfection purposes. (Muhammad *et al.*, 2004) surface sterilized the explants for 15 minutes with 50 per cent commercial bleach (Clorox 5.75% NaOCl) to which few drops of Tween-20 were added.

In vitro culture of banana :

Establishment of in vitro culture:

The isolated and properly surface sterilized explants were directly inoculated to each of the jam bottles containing 20 ml of MS medium supplemented with different concentrations of Auxins and cytokinins.The best medium for establishment of banana explant was supplemented with 3mg/lit. BAP, 0.2mg/lit. IAA with 30 mg ascorbic acid. Effect of different concentrations and combinations of phytohormones for establishment of *in vitro* explants of banana were observed in Table 2 .There was no any special effect of adenine sulphate so it was discarded in the next treatment.

Proliferation and subculture of in vitro shoots:

Effect of different concentrations and combinations of plant growth regulators on *in vitro* shoot proliferation from shoot tip explants of banana are shown in Table 3. There were lots of changes observed after 5,10 and 15 days.On the 5 days the sucker started greening and after 10 days unopened leaf whorls were observed, length of the explant was increased and gradual sprouting of shoot buds are observed (Fig. 1 a and b). This gave higher number of plants per explant. Maximum number of shoots in the combination of 5mg/lit. BAP, 0.5mg/lit. IAA and 30 mg ascorbic acid and 10 mg/lit. BAP, 0.5 mg/lit. IAA and 30 mg ascorbic acid. The ascorbic acid showed its effect as the blackening of the explant was lowered.After first and second culture the explant has shown very good result (Fig. 2 a and b).

Rahman *et al.* (2004); Habiba (1994) and Ali (1996) observed that hard ball like structure developed at the base of the shoot during shoot multiplication in MS media supplemented with 5.0 mg/lit. BAP. (Hwang *et al.*, 1984; Drew and Smith, 1990) reported that most common salt

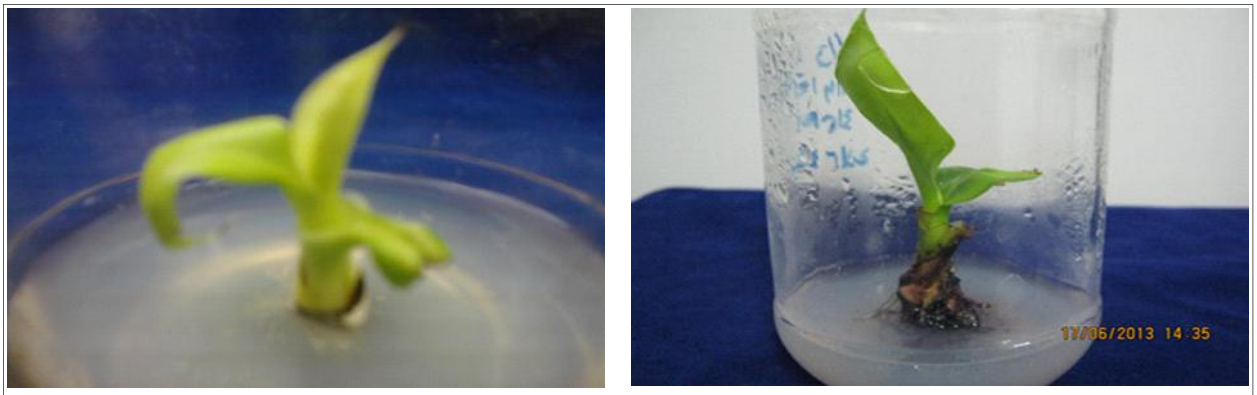
Table 1 : Effect of various treatments for sterilization of the banaa explant

Sterilant's concentration (%)	Duration (min)	Treatments											
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
$HgCl_2$(%)	2	0.01	0.01	0.01	0.1	0.1	0.1	-	-	-	-	-	-
	5	-	-	-	-	-	-	0.01	0.01	0.01	0.1	0.1	0.1
NaOCl (%)	5	20	30	40	20	30	40	-	-	-	-	-	-
	8	-	-	-	-	-	-	20	30	40	20	30	40
Ethyl Alcohol (%)	2	70	70	70	70	70	70	70	70	70	70	70	70
Contamination (%)		90	70	65	60	50	40	70	50	30	35	30	20

Table 2 : Effect of different concentrations and combinations of plant growth regulators for establishment of *in vitro* explants of banana

Name of the (MS) medium	BAP (mg/l)	IAA (mg/l)	Kinetin (mg/l)	Additives		No. of explants inoculated	Percentage of explants forming shoots (%)	No. of shoots per explant	Mean shoot length (cm)
				Adenine sulphate (mg/l)	Ascorbic acid (mg/l)				
E_1	1					6	50	1	6
E_2	1	0.5	1			5	60	1	10
E_3	1	0.5	1		30	3	67	2	5
E_4	1	0.5	1	100	30	4	60	1	5
E_5	2	0.2			30	5	60	2	9
E_6	2.5	0.25				5	40	1	8
E_7	3	0.2			30	4	75	3	7
E_8	4					6	60	1	7

mixture used for culture initiation of banana was the Murashige and Skoog (1962) media with some modifications.Assani et al. (2003) initiated cultures from anthers on MS medium containing vitamins of Morel supplemented with 500 mg/lit casein hydrolysate, 4.4µM BAP, and 2.3µ M IAA. (Hwang *et al*., 1984; Drew *et al*., 1989) used kinetin. Adenine sulphate was also added in the medium as a conducive agent to shoot initiation. In the current study there was no any special effect of kinetin and adenine sulphate. In this study Casein hydrolysate was not used. In this investigation BAP and IAA were the most effective phytohormones for the

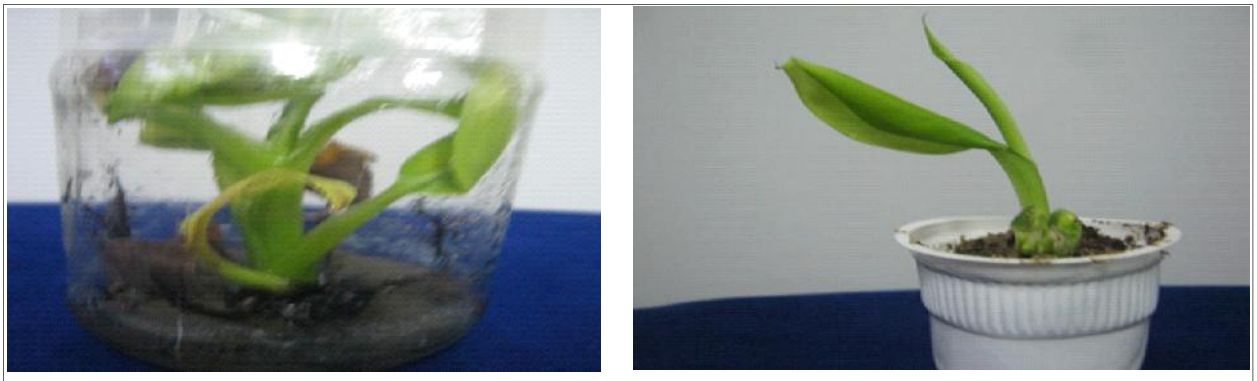


(a) **(b)**

Fig. 1 : Establishment of *in vitro* micro propagation of banana suckers inoculated on MS medium after a: 5 days of inoculation, b: 10 days of inoculation

(a) **(b)**

Fig. 2 : Proliferation of *in vitro* micro propagation of banana sucker inoculated on MS medium after a: the 1st subculture, b: the 2nd subculture



(a) **(b)**

Fig. 3 : a:Rooting of *in vitro* raised shoots of banana, b:Hardening of *in vitro* raised roots of banana plant

shoot initiation and proliferation. Mendes *et al*. (1999) revealed that highest shoot length (3.62 cm) was achieved when MS medium was supplemented with 1.5 mg/lit. NAA used 4.5 mg /lit BAP in MS medium.Abdullah *et al*. (1997) used MS medium with 20 µM BAP for shoot proliferation of dessert banana.Priyono (2001) reported cormlet production on medium supplemented with 5-20 mg/lit. (BAP) combined with 10-40 per cent sucrose or in the medium supplemented with 5-20 mg/lit. BAP combined with 5-20 mg/lit. ancymidol. Similarly, Noor-Aziah and Khalid (2002) used higher concentration of BAP during regeneration of *in vitro* banana plants from scalps and whole meristem.Venkatachalam *et al*. (2007) achieved direct shoot regeneration from leaf sheaths of silk banana when cultured on medium containing 22.4 µM BA. Bhagyalakshmi and Singh (1995) used MS medium with 8.9 M Benzyladenine and 0.9 M Indolebutyric acid during shoot culture of three cultivers of banana. Similarly, Okole and Schultz (1996) used MS medium along with 10 µM BAP and 1µM IAA for shoot Multiplication during culture of leaf segment from banana plants as an alternate approach for production of adventitious shoots and callus. Hwang *et al*. (1984) added 2 mg/lit. kinetin and 2 mg/lit. indole acetic acid in MS medium during meristem culture of banana and the population of buds was increased by 5 times per month. Wong (1986) compared Kinetin and 6-Benzylaminopurine (BA) along with Indole butyric acid (IBA) during *in vitro* multiplication of banana (*Musa* spp) and found that BA was more effective than kinetin.(Kulkarni *et al.,* 2006; Bakry *et al*., 2008) studied how media formulation and explant choice control the organogenic response. Dubois *et al*. (2009) explained *in vitro* Mutagenesis in Banana Improvement. Su *et al*. (2008) used ascorbic acid to control the lethal browning of tissue culture plantlets of banana.Jafari *et al*. (2010) explained the effect of benzyl aminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana). Rahman *et al*. (2004); Resmi and Nair (2007); Farahan *et al*. (2008) and Buah *et al*. (1998) described the effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures. North *et al*. (2012) stated that oxidized phenolic compounds may inhibit enzyme activity and result in the darkening of the culture medium and subsequent lethal browning of explants.

Rooting and hardening:

Root numbers varied with different concentrations of IBA and IAA. Significantly the best result means highest number of roots and greater root length were produced by 0.5 mg/lit. IAA + 0.5 mg/l IBA.The results on the effect of different concentration of IBA and IAA on root formation have been shown in the following (Table 4, Fig. 3 a and b). Meristem derived plantlets were

Table 3 : Effect of different concentrations and combinations of plant growth regulators on *in vitro* shoot proliferation from shoot tip explants of banana

Name of the (MS) medium	BAP (mg/lit.)	IAA (mg/lit.)	Ascorbic acid (mg/lit.)	No. of explant inoculated	Percentage of explant forming shoots (%)	No. of shoots per explant	Mean shoot length (cm)
P_1	4	0.2		4	75	1	5
P_2	4	0.2		6	50	2	6
P_3	4	0.2	30	5	80	4	6
P_4	5	0.2	30	4	75	3	4
P_5	5	0.5	30	5	80	5	7
P_6	5	0.5		6	60	2	6
P_7	7			2	50	2	5
P_8	7	0.5	30	4	75	4	6
P_9	10	0.5	30	5	80	5	7
P_{10}	10	1	30	5	60	2	5

Table 4 : Effect of different concentrations and combinations of phytohormones for rooting

Name of the (MS) medium	IAA(mg/lit.)	IBA(mg/lit.)	No. of roots	root length(cm)
RC-1	0.5	1.5	4	3
RC-2	1.0	0.5	3	2
RC-3	0.5	0.5	5	4

transferred to poly bags containing 1:1 (ground soil: cowdung) mixture after 7 days hardening in room temperature (28-30°C).

Summary and conclusion

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). The present investigation entitled. "Mass *in vitro* micro propagation of banana" was carried out to optimize the concentration and duration of different sterilants for explants surface sterilization and develop a suitable and efficient protocol for plant regeneration in banana through micro propagation.

The present investigation shows that among the different treatments tried out,the best sterilants for the surface sterilization of the explant of banana were 0.1 per cent $HgCl_2$ for 5 min followed by NaOCl (40 %, commercially available) solution for 8 min and 70 per cent ethyl alcohol for 2 min.The present study revealed that among the different phytohormones enriched medium,the best medium for establishment of banana explant was supplemented with 3mg/lit. BAP, 0.2 mg/lit. IAA with 30 mg ascorbic acid and the best medium for Proliferation of banana explant was supplemented with 5mg/lit. BAP,0.5mg/lit. IAA with 30 mg ascorbic acid and 10 mg/lit. BAP,0.5 mg/lit.IAA with 30 mg ascorbic acid. On the basis of observation made and results obtained it can be concluded that the *in vitro* cultivation of banana is of great importance as it yields a large number of plantlets within a short period of time.

REFERENCES

Abdullah, K., Khan, I.A., Siddiqui, S.H., Ahmed, M. and Siddiqu, K.A. (1997). *In vitro* culture of indigenous and exotic banana clones for maximum multiplication. *Pak. J. Bot.,* **29** : 143-150.

Al-Amin, Md., Karim, M. R., Amin, M. R., Rahman, S. and Mamun, A.N.M. (2009). In vitro micro propagation of banana (*Musa* spp). *Bangladesh J. Agril. Res*., **34**: 645-659.

Ali, A. S., Anum., Naveed, N., Majid, A., Saleem, A., Khan, U. A., Jafery, F.I. and Naz, S. (2011). Initiation, proliferation and development of micro-propagation system for mass scale production of banana through meristem culture. *African J. Biotechnol*., **10**:15731-15738.

Aquil, B., Arif, T.J., Sarin, N.B. and Haq, Q.M.R. (2012). Micro propagation and genetic transformation of banana for crop improvement and sustainable agriculture. *J. Crop Sci.,* **3** : 64-77.

Assani, A., Bakry, F., Kerbellec, F., Haicour, R., Wenzel, G. and Foroughi-Wehr, B. (2003). Production of haploids from another culture of banana [*Musa balbisiana* (BB)]. *Plant Cell Reports,* **21** : 511-516.

Banerjee, N. and Sharma, A.K. (1988). *In vitro* response as a reflection of genomic diversity in long-term cultures of Musa.Theor. *Appl. Gene*., **76**: 733-736.

Bakry, F., Assani, A. and Kerbellec, F. (2008). Haploid Induction: androgenesis in *Musa balbisiana*. *Fruits*, **63** : 45-49.

Bhagyalakshmi, N. and Singh, N.S. (1995). Role of liquid versus agar-gelled media in mass propagation and ex vitro survival in bananas. *Plant Cell Tiss. Org. Cult*., **42**: 71-73.

Buah, J. N., Kawamitsu, Y. and Murayama, S. (1998). Nursery growth of banana (*Musa* spp.) plantlets rooted on auxin-free and auxin-supplemented media. *Plant Production Sci*., **1**: 207-210.

Drew, R.A. and Smith, M.K. (1990). Field evaluation of tissue cultured bananas in southeastern Queensland. *Australian J. Experimental Agric*., **30** : 569-574.

Dubois, T., Coyne, D., Kahangi, E., Turoop, L. and Nsubuga, E.W.N. (2009). Endophyte-enhanced banana tissue culture technology transfer through public-private partnerships in Kenya and Uganda. *ATDF J*., **3**:18-24.

Farahan, F.H., Aminpoor, M., Sheidai, Z., Noormohammadi. and Mazinan, M.H. (2008). An improved system for in vitro propagation of banana (*Musa acuminate* L.) cultivars. *Asian J. Plant Sci.,* **7**:116-118

Gabriela, V. (2011). Effect of adenine sulfate (adso4) on the in vitro evolution of white clover variety (trifoliumrepens l.).*AnaleleUniversitatii din Oradea, ascicula Protectia Mediului.* , **17** : 203-210.

Habiba, U., Reja, S., Saha, M.L. and Khan, M.R. (2002). Endogenous bacterial contamination during in vitro culture of table banana: Identification and prevention. *Plant Tissue Cult.,* **12**: 117-124.

Hwang, S.C., Chen, C.L., Lin, J.C. and Lin, H.L. (1984). Cultivation of banana using plantlets from meristem culture. *Hort. Sci*., **19** : 231-232.

Jafari, N., Othman, R.Y. and Khalid, N. (2010). Effect of benzyl amino purine (BAP) pulsing on *in vitro* shoot multiplication of Musa acuminata (banana). *Asian J. Plant Sci. & Res*., **10** : 2446-2454.

Jalil, M., Khalid, N. and Othman, R.Y. (2003). Plant regeneration from embryo genic suspension cultures of *Musa*

acuminata cv. PISANG MAS. *Plant Cell, Tissue & Organ Culture*, **75**: 209-214.

Kalimuthu, K., Kumar, S.M. and Kumar, S.R. (2007). *In vitro* micro propagation of *Musa sapientum* L. (Cavendish Dwarf). *African J. Biotechnol*., **6** :1106-1109.

Kulkarni, V.M., Suprasanna P. and Bapat V.A. (2006). Regeneration through multiple shoots and somatic embryogenesis of a commercially important and endangered Indian banana Rajeli. *Curr. Sci*., **90** : 842-846.

Kahangi, E.M. (2010). The potential of tissue culture banana (*Musa* spp.) technology in Africa and the anticipated limitations and constraints. *Acta Hort. (ISHS*), **879**: 281-288.

Mendes, B.M.J., Flippi, S.B., Dementrio, G.B. and Rodriguez, A.P. (1999). A statistical approach to study the dynamics of Micro propagation rate using banana (*Musa* spp) as example. *PI. Cell Rep*., **18**: 967-971.

Muhammad, A., Hussain, I., Naqvi, S.M.S. and Rashid, H. (2004). Banana plantlet production through tissue culture. *Pak. J. Bot*., **36**: 617-620.

Nandwan, D., Zehr, U., Zehr, B.E. and Barwale, R.B. (2000). Mass propagation and ex vitro survival of banana cv. 'Basrai' through tissue culture. *Gartenbauwissenschaft.*, **65** : 237-240.

Noor-Aziah, J. and Khalid, N. (2002). Comparative Analysis of RegenerantsBetween Single and Naked Meristem (scalps) of Musa Acuminata Var. Berangan. *Asia Pacific J. Molecular Biol. & Biotechnol.*, **10** : 127-131.

North, J.J., Ndakidemi, P.A. and Laubscher, C.P. (2012). Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micro propagation of Strelitziareginae. *International J. Physical Sci.*, **7**: 638 - 646.

Okole, B.N. and Schultz, F. A. (1996). Micro-cross-sections of banana and plantain (*Musa* sp.): morphogenesis and regeneration of callus and shoot buds. *Plant Sci*., **116**:185-195.

Priyono (2001). Micro propagation of banana (*Musa paradisiacal*) through cormlet initiation by in vitro culture of apical meristem slices. *J. ILMU DASAR*, **2** : 41-48.

Rahman, M.Z., Nasiruddin, K.M., Al-Amin, M. and Islam, M.N. (2004). *In vitro* response and shoot multiplication of banana with BAP and NAA. *Asian J. Plant Sci*., **3**:406-409.

Resmi, L. and Nair, A.S. (2007). Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India. *Plant Cell Tissue Organ Cult.*, **88**: 333-338.

Sheidai, M., Aminpoor, H., Noormohammadi, Z. and Farahani, F. (2009). Genetic variation induced by tissue culture in Banana (*Musa acuminate* L.) cultivar Cavandish Dwarf Masoud. *Geneconserve*., **9** : 1-10.

Silva, A.D., Matssumoto, K., Bakry, F. and Souza, R.B.B. (1998). Plant regeneration from long term callus culture of AAA-group dessert banana.Pesq. *Agropec. Brasilia*., **33** : 1291-1296.

Venkatachalam, L., Sreedhar, R. and Bhagyalakshmi, N. (2007). Micro propagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regul*., **51**:192-205.

Vora, N.C. and Jasrai, Y.T. (2012). Natural and low-cost substitutes of synthetic pgr for micro propagation of banana. *CibTech. J. Biotechnol*., **2** :9-13.

W.H.K, C.C., Su, C. L. and Chen, C. P. (2008). Control of lethal browning of tissue culture plantets of banana of ascorbic. *Cult*., **96** : 137-141.

Wong, W.C. (1986). *In vitro* propagation of banana (*Musa* spp.): initiation, proliferation and development of shoot-tip cultures on defined media. *Plant Cell Tissue Organ Cult*., **6**: 159-166.