The Bioscan 8(3): 839-844, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IN VITRO SHOOT TIP CULTURE OF BANANA CULTIVAR *MEITEI HEI*

ROBERT LALRINSANGA* H.VANLALDIKI AND W.I MEITEI
Department of Horticulture, College of Agriculture, Central Agricultural University,
Imphal, Manipur - 795 004, INDIA
e-mail: robertlalrinsanga@yahoo.com

KEYWORDS
Micropopagation
Shoot-tip, *Meitei hei*
Banana, Culture

Received on :
01.02.2013

Accepted on :
31.05.2013

***Corresponding author**

ABSTRACT
Micropopagation is preferred over the conventional method of propagation in banana owing to its faster multiplication rate, uniformity in planting materials, production of disease-free planting materials, higher bunch weight, more fingers and hands and less variability in fruit size and shape. The apical meristem or shoot-tip culture is very efficient for rapid clonal micropopagation. Though abundant information on the micropropagation of banana is available for cultivars in India and abroad, there is only little or no information on micropropagation of *Meitei Hei*, a popular local variety of Manipur. Thus, study was undertaken during 2009-10 in the Tissue Culture Laboratory of the Department of Horticulture, CAU, Imphal on the in vitro shoot tip culture of Meiteti Hei banana to analyze its micropropagation potential. Results revealed that the highest multiple shoot induction was found in MS + 5mg1^{-1}BAP at 2.17 shoots while MS + 1 mg1^{-1}NAA + 0.2 mg1^{-1}BAP gave the longest regenerated shoots after 45 days of incubation. Highest number of roots was found in MS + 2 mg1^{-1}NAA. 98-100% survival was found in all the ex-vitro hardening media of sand, soil:FYM (2:1) and soil:vermicompost (2:1).

INTRODUCTION

Banana is one of the most important major fruit crops grown in India. Botanically, banana is a monocotyledonous herbaceous plant belonging to the section Eusuma under the family Musaceae (Purseglove, 1976). Almost every part of the banana plant is used some way or the other and it is rightly called "poor man's apple". It is probably the cheapest fruit available throughout the year. Banana occupies an important position in the Indian economy and it is the second most important fruit crop next only to mango. The Assam-Burma-Thailand region (now known as the Indo-Myanmar mega-biodiversity hotspot) is supposed to be not only the center of origin but also the centre of diversity (Simmonds, 1962). Of the different cultivars grown in Manipur, the local cultivar-*Meitei Hei* or *Hei* is the most popular and thrives well even under low temperature conditions. It has been in cultivation since time immemorial in Manipur, which lies well within the 'Indo-Myanmar mega biodiversity hotspot', the center of origin and centre of diversity of banana. *Meitei Hei* is famous for its sweetness and good desirable quality. The lamina of the leaf is used as substitute for dinner plates during festivals and marriage feasts and the pseudostem is also used as vegetable curry. It is a cold tolerant variety thriving well even at 12-15^{o}C. Though *Meitei Hei* is a household name which is synonymous with the word 'fruit' in the region, its commercial cultivation and marketing have not been exploited till date mainly due to lack of availability and required quantity of quality planting materials.

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 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). 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 and Zamora *et al*., 1986), little or no information is available on the micropropagation of *Meitei Hei*. Keeping in view these considerations, investigation was undertaken to study and analyze the micro- propagation potential of *Meitei Hei*.

MATERIALS AND METHODS

Prepareation of Media

MS (Murashige and Skoog, 1962) media supplemented with different and various combinations of plant growth regulators were used for shoot-tip culture. *Myo*-inositol and sucrose were added freshly. Plant growth regulators from stock solutions were added as per requirement. The pH of the solution was adjusted to 5.8 using 1N NaOH or 1N HCl and the final volume made up. After making up the final volume, the solution was

heated and agar @ 0.8 % was dissolved to make the media semi-solid. While heating, the media was stirred frequently with a glass rod or shook frequently for uniform heat distribution and to avoid frothing and boiling. After the agar was dissolved completely, the media was distributed into test-tubes (150 x 25 mm borosil tubes) or conical flasks (100,150, 250 ml volume). The tubes and flasks were plugged tightly with non-absorbent cotton wrapped with cheesecloth. The culture vessels containing media were sterilized by autoclaving at 15 p.s.i(121°C) for 20 minutes. After autoclave sterilization they were cooled at room temperature and then transferred to the inoculation room.

Culture conditions

The inoculated culture materials were kept in culture room maintained at 25 ± 2°C and 60-70 % RH under 12 hours light (intensity of 3000 lux, fluorescent tubes, Bajaj, 6500° K As., 40 watts) and dark cycle.

Isolation of shoot-tips from suckers

Shoot-tips along with adjacent corm tissues were carefully excised from the suckers and washed under tap water. The shoot-tips of about 1-2 cm size were then soaked in 2% Teepol for 5 minutes. After decanting Teepol, the shoot-tips were rinsed with distilled water.

Surface sterilization of shoot-tips

The isolated shoot-tips were surface sterilized by treating with 0.1 % w/v $HgCl_2$ for 15 minutes and then rinsed thrice with sterile distilled water to ensure complete removal of surface sterilant. All these operations were carried out under aseptic conditions in a laminar flow. The sterilized shoot-tips were placed in a sterile petri-dish. Some of the ensheating leaf primodia were systematically removed with the help of forceps and scalpel. Corm tissues were trimmed from the base as such superfluous tissues cause excessive blackening. At this stage, the explants of 1 cm consisted of shoot apical meristem covered with 2-8 leaf primordial and small basal corms. The removal of the outermost over arching leaves was done during sub culturing of the explants.

Induction of multiple micro-shoots, their growth and development and rooting.

Trimmed and sterilized explants were inoculated in MS media supplemented with different combinations and concentrations of growth regulators. Cultures were observed daily. Sub-culturing was done every 4 weeks to enhance response of shoot-tips to the various treatments. From the multiple micro-shoots induced in the multiplication stage, individual shoot buds of uniform height (approx.1cm) were cut and separated from the various multiplication media. Cutting of the multiple micro-shoots into individual shoot buds was done under laminar airflow with the help of sterilized forceps and blades and were subsequently transferred into the growth and development media. Plantlets of uniform height of 5cms with 2-3 leaves were selected for the experiment and the roots were trimmed under aseptic condition. The culture materials were kept in culture room maintained at 25 ± 2ºC and 60-70 % RH under 12 hours light (intensity of 3000 lux, fluorescent tubes, Bajaj, 6500ºK As., 40 watts) and dark cycle.

Hardening of plantlets

Plantlets with well-developed roots were subsequently transferred to hardening media. The hardening media consisted of half strength MS media without any growth regulators. After 25 days of incubation, the *in vitro* hardened plantlets were transferred to different potting mixtures of soil : vermicompost 2:1 (v/v), river-sand and soil : FYM 2:1(v/v) maintained inside green house conditions of 22-24°C and 90-95 % RH. The high humid condition was maintained with the help of over-head mists. Percentage of survival and growth rate was observed for the plantlets in the different potting mixtures.

Statistical analysis

The experimental design was completely randomized. The effects of treatments were tested by Analysis of Variance. Duncan's Multiple Range Test (DMRT) (Duncan, 1955) tested difference among means.

RESULTS AND DISCUSSION

Induction of multiple shoots

After 75 days of incubation, the number of multiple buds in all the media ranged from 0.11 in control to 2.17 numbers in MS + 5 mg l^{-1} BAP which was the highest. Wong (1986) also observed 2.6 shoots/budmass in the cultivar Lacatan (AAA) in MS + 5 mg l^{-1} BAP while Zamora *et al*. (1986) reported 3 shoot buds per explant after 6 to 8 weeks of culture in MS media supplemented with 5 mg l^{-1} BAP in banana cultivar Kanchkal (ABB). An average of 3.3 micro-shoots/explant was induced in the cultivar Monthan in similar media by Balakrishnamurthy and Sreerangaswami (1987). Successful shoot-tip multiplication of diploid (AA) 0304-02, triploid (AAA) Nanicao and tetraploid (AAAB) JV 42-29 in MS + 5.0 mg l^{-1} BAP was also reported by De-Oliveira *et al*. (1999).

The highest average length of the micro-shoots was observed in the media MS + 0.1mgl^{-1}NAA + 0.5 mgl^{-1}BAP (1.20cms). The same treatment also affected the highest number of leaves (4.17) and the highest number of roots (2.44). The lowest number of leaves and roots were found in the MS media alone *i.e.* control (Table 1).

Growth and development of multiple shoots

Varying response was observed in all the media tested for growth and development. MS media without growth regulators gave a minimal response and was the least effective. Hundred per cent response was observed in MS + 1.0 mgl^{-1}NAA and MS + 1.0 mgl^{-1} NAA + 0.2 mgl^{-1} BAP while the media MS + 0.5 mgl^{-1}NAA and MS + 0.5 mgl^{-1}NAA + 0.2 mgl^{-1}BAP gave a good response of 90% each. The media without any growth regulators gave a response of only 25% for growth and development (Table 2).

After 45 days of culture, the highest number of leaves was found in the media MS + 1.0 mgl^{-1} NAA + 0.2 mgl^{-1} BAP (3.40) while the least number was observed in MS + 1.0 mgl^{-1} NAA(1.54) amongst the hormonal treatments. The media

Table 1: Morphogenetic responses of the shoot tips of banana var. *Meitei Hei* cultured on MS media supplemented with different concentrations and combinations of Plant Growth Regulators after 75 DAI.

S. No.	Treatments	No. of micro-shoot per explant	Length of micro-shoots (cm)	No. of leaves per micro-shoot	No. of roots per micro-shoot
1.	MS (Control)	0.11^{d}	0.02^{f}	0.39^{d}	0.33^{c}
2.	MS + 0.5 mgl^{-1} BAP	0.50^{c}	0.16^{e}	1.11^{c}	0.44^{c}
3.	MS + 2.0 mgl^{-1} BAP	1.06^{b}	0.18^{e}	2.05^{b}	0.67^{c}
4.	MS + 5.0 mgl^{-1} BAP	2.17^{a}	0.68^{b}	2.17^{b}	1.06^{c}
5.	MS + 0.1mgl^{-1}NAA + 0.5 mgl^{-1}BAP	1.50^{b}	1.20^{a}	4.17^{a}	2.44^{a}
6.	MS + 0.1mgl^{-1}NAA + 2.0 mgl^{-1}BAP	1.39^{b}	0.41^{c}	1.89^{b}	1.28^{b}
7.	MS + 0.1mgl^{-1}NAA + 5.0 mgl^{-1}BAP	1.22^{b}	0.39^{c}	2.39^{b}	0.56^{c}
8.	MS + 0.5mgl^{-1}NAA + 0.5 mgl^{-1}BAP	1.28^{b}	0.31^{d}	2.22^{b}	0.78^{c}
	SE d ±	0.14	0.02	0.23	0.22
	CD (p = 0.05)	0.30	0.04	0.49	0.47

Table 2: Growth and development of multiple micro-shoots of banana var. Meitei Hei cultured in different modified MS media

Treatment	Treatment Composition	% of shoot buds responded for regeneration and rooting	Days required for root initiation	Length of regenerated shoot after 45 days of culture (cm)	No. of leaves per shoot after 45 days of culture (cm)	Average length of root (cm) after 45 days of culture	Average no. of roots per regenerated shoot after 45 days of culture	Remarks
MSR_0	MS(Control)	$25(30)^{c}$	41.40^{d}	1.61^{c}	0.20^{d}	0.10^{d}	0.40^{c}	Very low response
MSR_1	MS + 0.5 mgl^{-1}NAA	$90(81)^{b}$	11.59^{c}	2.22^{b}	1.59^{c}	1.00^{c}	3.10^{b}	Slow regeneration and rooting
MSR_2	MS + 0.5 mgl^{-1}NAA + 0.2 mgl^{-1} BAP	$90(81)^{b}$	11.49^{c}	5.90^{a}	2.40^{b}	1.02^{c}	3.00^{b}	Proper regeneration but slow rooting
MSR_3	MS + 1.0 mgl^{-1} NAA	$100(90)^{a}$	7.29^{b}	2.18^{b}	1.54^{c}	1.94^{b}	8.60^{a}	Minimal growth but early rooting
MSR_4	MS + 1.0 mgl^{-1} NAA + 0.2 mgl^{-1} BAP	$100(90)^{a}$	6.93^{a}	6.20^{a}	3.40^{a}	3.40^{a}	8.30^{a}	Early rooting and proper regeneration
SE d±	8.05	0.16	0.15	0.29	0.13	0.24	-	
CD (p=0.05)	16.79	0.33	0.31	0.60	0.27	0.52	-	

Table 3. Response of regenerated plantlets to root induction in different modified MS media

Treatment	Treatment composition	Days to initiation	No. of roots per explant	Average length of roots per explant	Remarks
MSM_0	MS (Control)	4.55^{a}	2.11^{f}	1.09^{c}	Mild response
MSM_1	MS + 0.1 mgl^{-1} NAA	13.78^{e}	5.67^{d}	1.33^{c}	Uniform growth
MSM_2	MS + 0.5 mgl^{-1}NAA	11.22^{d}	7.33^{c}	1.67^{b}	Unequal growth
MSM_3	MS + 1.0 mgl^{-1}NAA	10.99^{d}	8.89^{b}	2.22^{a}	Roots are long and thick
MSM_4	MS + 2.0 mgl^{-1}NAA	9.77^{c}	15.67^{a}	1.22^{c}	Profuse rooting with thin, small and hairy roots.
MSM_5	MS + 1.0 mgl^{-1}NAA + 0.2 mgl^{-1} BAP	5.44^{b}	3.67^{e}	0.61^{d}	Early root initiation, but slow increase in length and numbers.
SE d±		0.24	0.24	0.14	
CD (p = 0.05)		0.52	0.52	0.31	

Nos. of explants inoculated in each treatment: 18, DAI: Days After Inoculation, Figures in parenthesis are Arc Sin $\sqrt{\text{Percentage}}$,

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. Effect of different potting media on the survival and average increase in height of banana cv. *Meitei Hei*

Treatment	Survival	Average increase in height (cm)
River Sand	100	1.58
Soil : FYM (2:1)	98	3.50
Soil : Vermicompost(2:1)	99	4.11
S.E d ±	-	0.09
C.D (p = 0.05)	-	0.22

without any growth regulators (control) gave a minimal leaf number of 0.20 only. Profuse and early rooting was observed in MS + 1.0 mgl^{-1} NAA and MS + 1.0 mgl^{-1} NAA + 0.2 mgl^{-1} BAP though the average root length was the highest in MS + 1.0 mgl^{-1} NAA + 0.2 mgl^{-1} BAP. Days to rooting were comparatively slower in the media with low auxin concentration.

Various other researchers (Cronauer and Krikorian, 1984; Damasco and Barba, 1984; Jarret *et al*., 1985 and Sandoval, 1995) have also obtained good results for length of regenerated shoot, number of leaves and length of roots in MS media + 1mgl^{-1} NAA and 0.2 mgl^{-1} BAP and are of the opinion that lower cytokinin induces growth and elongation of buds while high auxin concentration induces rooting.

Rooting of regenerated plantlets

Root initiation and development was observed in all the media containing different concentrations of NAA, and also in the MS media alone and in the media containing a combination of NAA and BAP. Bekheet and Saker (1999) reported the superiority of NAA over IAA and IBA in the *in vitro* rooting of banana plantlets *cv*. William, Grande Naine and Maghraby

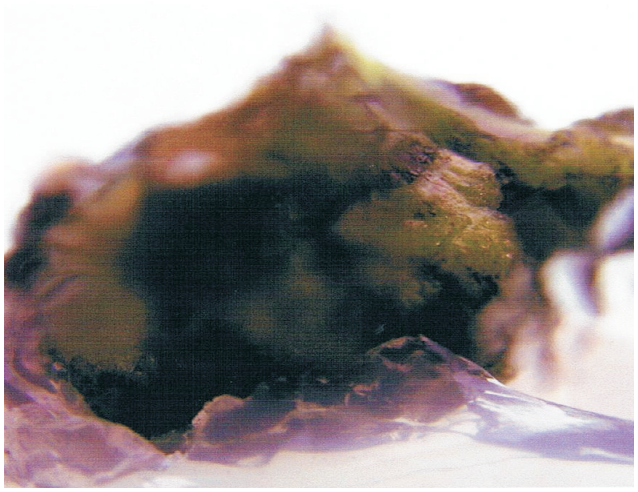

Figure 1: Swelled shot-tip in MS + 5 mgl^{-1} BAP 30 DAI

Figure 2: Induced multiple buds in 5 mgl^{-1} BAP 75 DAI

Figure 3: Regenerated plantlets in MS + 1 mgl^{-1} + NAA + 0.02 mgl^{-1} BAP 46 DAI

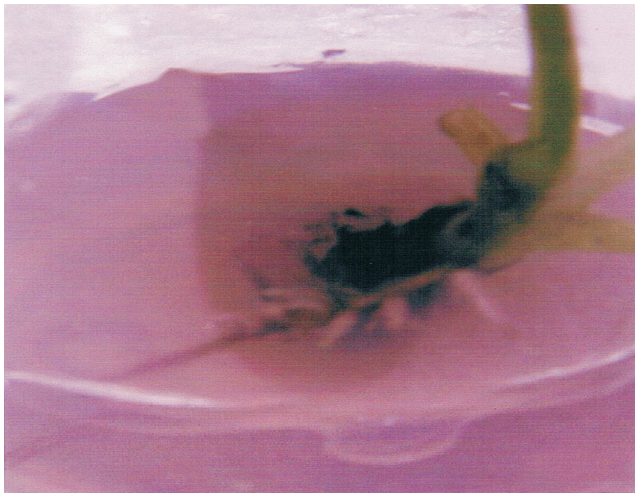

Figure 4: Induced roots in 1 mgl^{-1} NAA 25 DAI

Figure 5: *Ex vitro* plantlent hardening in sand culture

Figure 6: *Ex vitro* hardened plantlet transferred to soil and FYM potting mixture

while Ahsan *et al*. (1998) reported that the best response was achieved in hormone free MS media for table banana (*Musa sapientum*). The present finding of root induction in hormonal combinations confirms the findings of Babylatha *et al*. (1997) who achieved 100 % rooting in full or half MS media supplemented with various combinations of IBA, NAA and IAA.

All the media tested induced rooting within 14-15 days from the day of inoculation. The shortest days to root initiation of 4.55 days was observed in MS media that was devoid of any growth regulators. (Table 3). The media MS + 2.0 mg1^{-1}NAA gave the highest number of roots (15.67).The higher root induction in the said media is probably due to the requirement of auxin in very low concentrations as earlier studies have shown that the use of 1 ìM NAA l^{-1} was found sufficient for *in vitro* rooting of Dwarf Cavendish, Grande Naine, Petit Nain, Poyo, Williams and Basrai (Gubbuk and Pekmezci, 2001). Jambhale *et al*. (2001) also reported the successful *in vitro* rooting of Basrai (AAA), Nendran (AAB), Lal Kela (AAA) and Safed Velchi (AB) in MS media supplemented with 3 mg l^{-1} NAA while Acharjee (2004) reported that rooting of Bhimkhol, Malbhog, Kanchkal and Jahaji was optimum within 10-12 days in MS + 0.2 mg l^{-1} NAA. MS media alone without growth regulators gave the lowest number of roots (2.11) while the highest average length of roots was found in the media MS + 1.0 mg1^{-1}NAA (2.22 cm).

The highest number of roots was found in the media MS + 2.0 mg1^{-1}NAA with 15.67 roots per plantlet. Earlier studies have shown that the use of 1 ìM NAA l^{-1} was found sufficient for *in vitro* rooting of Dwarf Cavendish, Grande Naine, Petit Nain, Poyo, Williams and Basrai (Gubbuk and Pekmezci, 2001). Jambhale *et al*. (2001) reported the successful *in vitro* rooting of Basrai (AAA), Nendran (AAB), Lal Kela (AAA) and Safed Velchi (AB) in MS media supplemented with 3 mg l^{-1} NAA while Acharjee (2004) also reported that rooting of Bhimkhol, Malbhog, Kanchkal and Jahaji was optimum within 10-12 days in MS + 0.2 mg l^{-1} NAA.

MS + 1.0 mg1^{-1}NAA affected the second highest number of roots with 8.89 roots per plantlet. Of all the media tested, MS media alone without growth regulators gave the lowest number of roots with an average of only 2.11 roots per plantlet. Amongst the media with growth regulators, the media MS + 1.0 mg1^{-1} NAA + 0.2 mg1^{-1} BAP gave the lowest number of roots with 3.67 roots per plantlet. Hence, there were only marginal differences in the number of roots with control though there was a significant difference between the two. The highest average length of roots was found in the media MS + 1.0 mg1^{-1}NAA (2.22 cm) followed by the media MS + 0.5 mg1^{-1}NAA with 1.67 cm per plantlet. Length of roots in the MS media alone, MS + 0.1 mg1^{-1}NAA and MS + 2.0 mg1^{-1}NAA were at par, the root length ranging from 1-1.5 cm.

Hardening of fully developed rooted plantlets

The fully developed plantlets were in-vitro hardened for 25 days in half strength MS media, then transferred to different potting media under Green House conditions. After 30 days, the plantlets in the different potting media were analyzed for percentage of survival and average increase in height. Silva *et al*. (1998) have also successfully pre-acclimatized the rooted plantlets of *cv*. Pioneira for 30 days in a Green House under controlled conditions of temperature and irrigation.

A survival of 98-100% was observed in all the treatments with 100 % survival in river-sand. Some of the findings of earlier workers indicated a survival percentage ranging from 80-100 when banana rooted plantlets were transferred to *ex vitro* hardening media under Green House or Shade House conditions (Palai and Das, 2002 ; Molla *et al*., 2004 and Acharjee *et al*., 2004). Sharma *et al*. (1997) also reported 97% survival of *in vitro* developed Dwarf Cavendish plantlets in sand culture while only 2% loss was observed during the acclimatization of Nanicao and Grande Naine in polythene bags containing equal proportions of organic manure : soil : sand (De-Oliveira and De- Oliveira-e-Silva, 1997).

The highest average increase in height was found in the potting media containing soil : vermicompost (2:1,v/v) with an increase in height by 4.11 cms after 30 days. This finding partially corroborates with the finding of Saindane *et al*. (1999), who reported that out of the different potting media of soilrite, vermicompost, leaf-mould, river sand and press-mud cake, vermicompost effected the maximum growth of stems, leaves and roots in the plantlets of *cv*. Grande Naine.

REFERENCES

Acharjee, S., Barooah, M. and Deka, P.C. 2004. *In vitro* propagation of four Musa spp. of the North-East Region of India. *Ann. Biol.* **20(1):** 1-6.

Ahsan Habib, Jahan, M.A.A. and Hossain, M.T. 1998. Acceleration of the process of *in vitro* root development in *Musa sapientum* var. Sagar. *Bangladesh J. Sci. Ind. Res.* **333**: 341-344.

Babylatha, A.K., Patel, B.M. and Shah, R.R.1997. *In vitro* propagation studies on banana *cv*. Basrai. *J. Appl. Hort.* Navsari. **3(1-2):** 12-22.

Balakrishnamurthy and Sreerangaswami, 1987. *In vitro* propagation of bananas. BANANA Taxonomy, Breeding and Production Technology. p. 91.

Bekheet, S.A. and Saker, M.M.1999. Rapid mass micropropagation of banana. *Bull. Nat. Res. Centre* Cairo. **24(2):** 221-232.

Cronauer, S.S. and Krikorian, A.D. 1984. Multiplication of Musa from excised stem tips. *Ann. Bot.* **53:** 321-328.

Damasco, O.P. and Barba, R.C. 1984. *In vitro* culture of Saba (Musa sp. *cv*.Saba BBB). *Phil. Agric.* **67:** 351-358.

De Oliveira, R.P, Silveira, D.G, De Oliveira, S.S and De Silva, K.M. and Vilarinhos, A.D. 1999. Evaluation of micropropagation of diploid, triploid and tetraploid banana genotypes, using a commercial protocol. *Revista Brasileira de Fruticultura.* **(3):** 269-273.

De-Oliveira,R.P. and De-Oliveira,S.S.1997. Evaluation of commercial microprogation for banana. *Pesquisa-Agropecuaria-Brasileira.* **32(4):** 415-420.

Dore Swamy, R., Srinivasa Rao, N.K. and Chacko, E.K. 1983. Tissue culture propagation of banana. *Sci. Hort.* **18:** 247-252.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics.* **11:** 1-42.

Gubbuk, H and Pekmezci, M. 2001. The effect of different hormone types and concentrations on propagation of different banana clones by meristem culture. *Ziraat Fakultesi Dergisi, Akdeniz Universitesi,* **14(1):** 127-137.

Jambhale, N.D., Patil, S.C., Jadhav, A.S., Pawar, S.V. and Waghmode, B.D. 2001. Effect of number of subcultures on *in vitro* multiplication

of four banana clones. *Infomusa*. **10(1):** 38-39.

Jarret, R.L., Rodriguez, W. and Fernandez, R. 1985. Evaluation, tissue culture propagation and dissemination of 'Saba' and 'Pelipita' plantain in Costa Rica. *Sci. Hort*. **25:** 137-147.

Kwa, M. and Ganry, J. 1989. Agronomic use of banana vitro plants. Fruits (Paris) *Special Issue,* (Fr, 17 ref., 2pl. Presented at the annual seminar of IRFA/CIRAD on banana & plantains, Montpellier.4-8 Sep.,1989), IRFA, 75116 Paris, France. pp. 107-111.

Molla, M.M.H., Khanam, M.d., Khatun, M.M., Al Amin, M. and Malek, M.A. 2004. *In vitro* rooting and *ex vitro* plantlet establishment of BARI banana 1 (Musa sp.) as influenced by different conc. of IBA (indole 3-butyric acid). *Asian J. Plant Sci*. **3(2):** 196-199.

Murashige, T. and Skoog, F. 1962. *Physiol. Plant*. **15:** 473.

Nandi, M., Joshi, V.J. and Chaudhari, A.N. 1998. Field evaluation for growth and yield parameters of banana (Musa spp.) derived from tissue culture and conventional planting material. *Indian J. Agrc. Sci*. **68(10):** 684-686.

Palai, S.K. and Das, A.B. 2002. Large scale propagation of *Musa balbisiana cv*. Muguni through *in vitro* techniques. Proceedings of the state level seminar on advances in production of quality planting materials of horticultural crops, 6-7 Sept., 2002. Orissa Horticultural Society, Bhubaneshwar, India. pp. 133-136.

Purseglove, J.W.C.1976. Tropical Crops: Monocotyledons. Longmans, N.Y. pp. 273-283.

Saindane, P.V., Baviskar, M.P., Bhalsing, S.R., Teli, N.P., Pathak, S.S., Singh, P., Maheshwari, V.L., Reddy, S.M., Rao, D. and Vidyavati, 1999. Cost effective hardening of tissue culture grown banana (*Musa paradisiaca*) var. Grande Naine plants. Perspectives in biotechnology, Proceedings of a national symposium, Warangal, India, 26-27, February 1999. 2001. pp. 133-138.

Sandoval, J.A. 1995. Micrpopagation de Musaceas. *ASBANA*, Costa Rica, p.23.

Sharma, G.L., Tiwary, B.L. and Pandey, S.D. 1997. Rapid *in vitro* mass propagation of banana and changes in bio-chemical constituents at various cultural stages. *Indian J. Hort*. **54(2):** 128-131.

Silva, de., S.O, Oliveira, R.P, Matos, de., A.P Silva, N.S.P, Prazere, A.G, Oliveira, C.A.P, Galan,S.V. 1998. Development of pre-acclimatized banana plantlets obtained from tissue culture. *Acta Hort*. **490:** 195-199.

Simmonds, N.W.1962. The evolution of banana. Longmans, London.

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

Zamora, A.B., Barba, R.C. and Damasco, O.P. 1986. Status and prospects of tissue culture research on bananas. In: Banana and plantation: Resarch and Development. Umali, B.E. and Lantican, C.M. (eds.). *Proc. Inter. Workshop,* Davao, Philippines, Feb. 25-27, 1985. pp. 78-88.