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EFFECT OF PERMANENT AND TEMPORARY IMMERSION SYSTEMS ON BANANA MICRO-PROPAGATION

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Abstract

For the establishment of a micro-propagation protocol for banana (*Musa* spp.) cv Basrai, meristematic stem cuttings were used as an explant. A number of cultures were maintained on MS medium supplemented with various auxins and cytokinins, of which a combination of IAA and BA for organogenesis and BA only for shoot induction/multiplication were considered as good agents for *In-vitro* propagation of banana. Micro-propagation efficiency was significantly (P >0.005) increased, when organogenesis was carried out by culturing on MS medium supplemented with 10.0 µM BA; 15.0 µM IAA and solidified with 3.60 g/L phytagel for 3-weeks, while shoot induction (1.0 g/L phytagel) and its multiplication (2.0 g/L phytagel) on MS medium supplemented with 10.0 µM BA for 2 and 3-weeks respectively. 17.65±0.50 plantlets per micro-stem cutting were developed through this protocol. Among others, in one medium (6.0 µM TDZ and 4.0 µM NAA or/and 10.0 µM BA) callus formation was observed but later on cultures proceeded to death, instead of multiplication. The phenolic oxidation was inhibited through the addition of L-cystein (30.0 mg/L) in each culture. Roots developed within 2-weeks, by culturing on $\frac{1}{2}$ MS basal medium supplemented with IBA (0.1 mg/L). Through this protocol, complete and normal micro-propagated plantlets were obtained within 2-3 months.

Introduction

Banana is one of the most important fruit crop grown all over the world. It provides a valuable source of income through local and international trade (Frison *et al*., 1997). Presently, world production reaches to approximately 40 tons/ha (Anon., 2005). Cultivated banana (3n) were derived from two diploid (2n) parent genomes of the genus *Mus*a, *M. acuminata* (Malaysia) and *M. balbisiana* (India) (Stover & Simmonds, 1987; Simmonds, 1962; Georget *et al*., 2000). However, expansion of banana production is limited, because of the shortage of healthy plant material availability to the farmers. The transmission of harmful insects, nematodes and viral disease by field-grown suckers has prompted interest in the use of aseptic culture techniques. Such disorders are reducible and/ may be eliminated by aseptic plant propagation.

With the increasing demand and vast export potential coupled with the farmers desire to grow *In-vitro* propagated banana on a large area are becoming increasingly important in planting material for rapid multiplication of economically important commercial varieties (Roux *et al*., 2001; Ray *et al*., 2006). So *In-vitro* propagation, appears to be an attractive system for banana, which makes it possible to get plantlets free from insects, bacteria and other micro-organisms (Krikorian & Cronauer, 1984; Ma & Shii, 1972; Vuylsteke, 1998) to fulfill farmer's demands.

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High production costs generally limit the commercial use of *In-vitro* micro-propagation because of its low efficiency. However, using liquid medium is considered to be the ideal solution for automation and reducing production costs (Be & Debergh, 2006). Meanwhile, the use of liquid media can be responsible for other problems such as asphyxia and/ hyper-hydricity (Etienne & Berthouly, 2002), causes to induce somewhat abnormalities in the developing plantlets. The prevention of such deficiencies is possible by using specific medium solidification condition for a specific stage of the micro-propagating plantlets (Alvard *et al*., 1993; Escalona *et al*., 1999).

The present work, suggests a rapid multiplication protocol for banana cv. Basrai from meristematic stem tips, by using a medium with optimized concentration of the auxins/cytokinins either through temporary immersion system and/or permanent immersion system (medium solidification). Our findings may be helpful for the establishment of banana micro-propagation techniques to produce rapid and clean clones. It may be of great value for the future research studies.

Materials and Methods

Four young banana (*Musa* spp.) cv Basrai plants were selected for use as an experimental material. Meristematic stem tips were excised from young suckers (Hwang *et al*., 1984). They were surface disinfected by washing with ethanol (90%) for 1 min. and then stirred in 20% commercially available robin bleach [5.25% (v/v) NaOCl] for 30 min., afterwards they were rinsed with sterile distilled water for 3 times in the laminar air flow cabinet .

Shoot tips (3-4 mm) were isolated aseptically and cultured for organogenesis on MS basal medium (Murashige & Skoog, 1962); B5 vitamins (Gamborg *et al*., 1968); 3% sucrose medium supplemented with MS_0 to MS_{2l} or without MS_m, L-cystein (30.0μM) and various combinations of different auxins and cytokinins for 3 weeks (Table 1). After organogenesis, the explants were sub-cultured in 2 ways, a: Refreshed on their respective medium (Table 1-B_{1a}); b: Organogenised explants from MS_{2j} medium were sub-cultured on all of these media (Table 1-B_{2a}).

Micro-propagation efficiency is dependent on the medium solidification. By using phytagel, three different solidification conditions such as routinely used tissue culture system (3.60 g/L phytagel); temporary immersion system (2.0 g/L phytagel) and permanent immersion system (1.0 g/L phytagel) were maintained during organogenesis, shoot induction and than their multiplication. These cultures were also interchanged at different developmental steps of the micro-propagating plantlets (Table 2-Ja & Jb). The mineral composition of each culture was same, while difference was solidification level of the medium (Roels *et al*., 2005).

pH was adjusted to 5.7-5.8 before autoclaving at 121°C and 15lbs for 15 min. Each culture was maintained with seven replicates at 25 ± 2°C under the light conditions with intensity of ~2000 lx provided by growth chamber with 18/6 h day and night photoperiod.

The established cultures on shoot induction medium were routinely transferred after every 3 weeks by subdividing bulky mass of plantlets into a number of micro cuttings. These micro cuttings of the micro-propagated clusters were sub-cultured for 4 times on the same medium (shoot multiplication). It's further sub-culturing (after 4^{th}) on the same medium caused abnormalities. These cultures have to be refreshed by taking new explants from the open field grown plants.

Table 1. Optimization of the micro-propagation efficiency in banana (*Musa* spp.) cv Basrai under different hormonal combinations.

Parameters	MS_0 Control	MS_{2a} Control L-cyst	MS_{2b} BA Kn L-cyst	MS_{2c} BA Kn IAA L-cyst	MS_{2d} BA Kn IAA TDZ L-cyst	MS_{2e} TDZ Kn IAA L-cyst	MS_{2f} TDZ IAA L-cyst	MS_{2g} TDZ L-cyst	MS_{2h} TDZ Kn L-cyst	MS_{2i} Kn IAA L-cyst	MS_{2j} BA IAA L-cyst	MS_{2k} BA TDZ L-cyst	MS_{2l} BA L-cyst	MS_{2m} BA
A. Organogenesis (3-weeks cultures) in the explants isolated from young banana plants from open field.														
a. Explant proliferation (g)	0.05	0.10	1.90	3.25	1.70	1.32	064	0.76	1.85	1.23	0.08	3.65	4.50	1.95
B_1: Each culture was refreshed on their respective medium after 4-weeks, for the purpose to develop shoots in explants.														
a. Number of shoots/explant	-	-	-	-	-	-	-	-	-	-	3.25	-	-	-
B_2. Micro-propagation (4-weeks culture) in the organogenised explants taken from MS_{2j} medium after 3-weeks.														
a. Number of shoots/explant	-	-	1.96	2.21	-	-	-	-	-	-	2.32	-	3.05	2.69
b Shoot height (cm)	-	-	1.36	1.76	-	-	-	-	-	-	5.31	-	3.42	3.35
c. Pseudostem diameter (cm)	-	-	0.26	0.23	-	-	-	-	-	-	1.41	-	1.12	1.08
C. Rooting on ½ MS_0 and with 0.01 and 0.10 mg/L IBA in the 4-weeks old micro-propagated plantlets (4^{th} sub-culturing) after 2-weeks.														
D. Rooting (%)														
a. 0.00mg/L IBA	-	-	0.00	0.00	-	-	-	-	-	-	8.12	-	19.82	5.27
b. 0.01mg/L IBA	-	-	19.68	12.14	-	-	-	-	-	-	37.28	-	55.65	45.19
c. 0.10mg/L IBA	-	-	58.62	62.38	-	-	-	-	-	-	68.92	-	85.58	80.56

Control: MS_0 (Murashige & Skoogs basal medium, B5 Vit, 3% Sucrose); **BA:** 10.0 µM; **Kn**: 4.0 µM; **IAA**: 15.0 µM; **TDZ**: 6.0 µM; L-cystein: 30.0 µM

Table 2. Different culturing conditions, used during the optimization of micro-propagation efficiency in banana (*Musa* spp.) cv Basrai.

#S	Culturing systems	Organogenesis media (MS_{2j}) 3-weeks culture	Shoot Induction media (MS_{21}) 2-weeks culture	Shoot multiplication media (MS_{21}) 4-weeks culture	# of shoots/ (cm) explant	Shoot height (cm)	Pseudostem diameter (cm)	*Root induction (%)
a.	Routinely used cultures (S)	3.60 g/L Phytagel	3.60 g/L Phytagel	3.60 g/L Phytagel	2.87±1.50	5.21±2.20	0.52±0.05	70.25±1.75
b.	S to P (After organogenesis)	-	1.00 g/L Phytagel	1.00 g/L Phytagel	5.47±0.80	2.13±1.85	0.38±0.60	10.92±1.50
c.	S to T (After organogenesis)	-	2.00 g/L Phytagel	2.00 g/L Phytagel	4.32±1.20	3.89±0.95	0.45±1.00	20.28±1.35
	Mean values for a, b & c				5.0925***	7.1632***	0.0147^{ns}	3043.228***
d.	Temporary immersion system (T)	2.0 g/L Phytagel	2.00 g/L Phytagel	2.00 g/L Phytagel	3.51±1.25	4.85±1.21	0.46±1.50	30.82±2.21
e.	T to S (After organogenesis)	-	3.60 g/L Phytagel	3.60 g/L Phytagel	1.25±2.00	5.52±1.70	0.57±1.28	40.67±1.80
f.	T to P (After organogenesis)	-	1.00 g/L Phytagel	1.00 g/L Phytagel	3.65±0.80	3.08±1.25	0.41±1.25	12.25±1.75
	Mean values for d, e & f				5.744**	4.7677***	0.0201*	324.7819***
g.	Permanent immersion system (P)	1.0 g/L Phytagel	1.00 g/L Phytagel	1.00 g/L Phytagel	1.62±1.34	3.31±0.92	0.34±0.80	2.10±2.50
h.	P to S (After organogenesis)	-	3.60 g/L Phytagel	3.60 g/L Phytagel	3.08±1.70	4.03±1.85	0.44±0.95	20.32±1.28
i.	P to T (After organogenesis)	-	2.00 g/L Phytagel	2.00 g/L Phytagel	2.36±1.20	3.45±0.95	0.37±0.62	5.18±1.89
	Mean values for g, h & I				3.2887**	0.4372^{ns}	0.0079*	317.4379***
ja.	Optimized protocol (Fig. 1)	3.60 g/L Phytagel	1.00 g/L Phytagel	2.00 g/L Phytagel	17.65±0.50	3.42±0.42	0.52±0.60	89.90±0.48
	Mean values for a, d, g & ja				169.4626***	208431***	0.0216*	4779.371***
jb.	Optimized protocol	3.60 g/L Phytagel	2.00 g/L Phytagel	1.00 g/L Phytagel	19.71±1.29	2.28±0.80	0.36±1.20	60.25±0.95
	Mean values for a, d, g & jb				218.8064***	5.5905***	0.0216*	168.0027***

*The medium composition, used for root induction was same as given in Table 1-D_c

Before each sub-culturing, at the micro-propagation stage, the number of shoots per explant (determined by counting the number of shoots/explant) and average shoot length (cm) were measured. Pseudostem diameter (cm) was also measured from its starting point after transversely cutting with scalpel. The root induction in the shoot cuttings was carried out by culturing onto ½ MS basal medium supplemented various concentrations of IBA (Table 1-D_c).

Statistical analysis for all parameters collected during this experiment was computed by using a *COSTAT* computer package (*CoHort Software*, Berkeley, USA).

Results

In order to establish an efficient *In vitro* micro-propagation system for banana (*Musa* spp.) cv Basrai, fresh meristematic shoot cuttings were cultured on MS basal medium (initial culture) supplemented with a number of combination of different cytokinins and auxins (Table 1). A number of cultures were maintained for the purpose to induce a mode/origin (organogenesis) in the cultured explants for shoot induction. After 3-weeks, explant proliferation was measured, which was increased on MS_{2k} medium supplemented with only BA, while any detectable proliferation was not observed on MS control and MS_{2f} (TDZ, IAA) media. Culture blackening was observed on MS_{2m} (without L-cystein) medium. Callus formation was observed in the medium supplemented with NAA and TDZ with/without BA; such developing tissues were not able to survive for much time (~4-weeks) and ultimately go to death.

For the purpose to find out which media is suitable for shoot induction: a) all cultures were refreshed on their respective media. 3.25 shoots were developed only on MS_{2ja} medium (Table 1-B_{1a}) after 4-weeks. b) Explants from MS_{2j} (Fig 2a) were also sub-cultured on each media. Maximum numbers of plantlets were observed on MS_{2l} within 4 week (Fig 2b) than on MS_{2m} and on others, after 5^{th} and 6^{th} weeks respectively (Table 1- B_{2a}).

The number of shoots/explant was counted, before their sub-culturing. Maximum shoots were observed on MS_{2l} followed by MS_{2m} and MS_{2i} (Table 1), however abnormal but low in numbers also developed on MS_{2b} and MS_{2c}. On MS_{2m} medium, normal plantlets were also developed but could not survive after 2^{nd} sub-culturing, because of culture blackening, which is probably due to phenolics oxidation.

The shoot height and the pseudostem diameter of the developing plantlets were also measured (Table 1). Both of them were maximum in MS_{2j} medium with low numbers of shoots, which is opposite to MS_{2l} medium. A correlation among the shoot height and pseudostem diameter with the numbers of micro-propagated plantlets per explant was observed. Maximum pseudostem diameter was in the plantlets which have highest plant height which decreased with the decrease in plant height, but the numbers of shoots per explant increased (Table 1- $B_{2b\ \&\ c}$).

To know the effect of medium solidification (physical conditions) on the micro-propagation efficiency in banana, the explants were organogenised by culturing on three different culture systems for 3-weeks i.e. routinely used tissue culture system (S) MS_{2j} (3.60 g/L phytagel), temporary immersion system (T) MS_{2j} (2.0 g/L phytagel) and permanent immersion system (P) MS_{2j} (1.0 g/L phytagel) (Table 2a, d & g), then were sub-cultured not only on the same medium but also one on two others. Two additional culture systems (Table ja & Jb) were also maintained by adding two different solidification conditions, one for shoot induction and 2^{nd} for shoot multiplication (Table 2). A clear cut difference was found in their growth rate and physical appearance in the micro-propagating plantlets (Table 2).

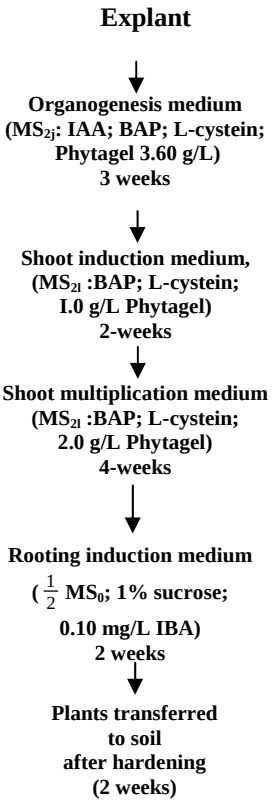

Fig. 1. A schematic representation of the optimized protocol (Table 2-ja) for the micro-propagation of the banana (*Musa* spp.) cv. Basrai.

Maximum numbers of shoot (p>0.005) were observed on the culture, which was sub-cultured from routinely used culture system (S) to permanent immersion system and than to temporary immersion system (Table 2-ja; Fig 3d). However, the maximum shoot height (p>0.005) was observed on the medium, which was sub-cultured from the temporary culture system (T) to routinely used culture system (S), where significant (p>0.5) increase in pseudostem diameter was also measured (Table 2-e). Somewhat similar parameters were also measured on routinely used culture, while the difference was only that the numbers of shoots per explant were non-significantly reduced (Table 2). The plantlets, which were propagated from solidified to solidified and permanent to permanent immersion system showed many drawbacks such as producing high levels of nitrifications, meristematic rhizome growth and many abnormal buds that were not suitable for banana micro-propagation purpose (Fig. 3a & c). The maximum numbers of shoots with moderate shoot height (p >0.005) were observed on optimized protocol (Table 2Ja), which were developed from the solidified (organogenesis) to permanent immersion system and then to the temporary immersion system (Fig. 1), while similar results were also noted on jb culture (Table 2) but rooting efficiency was very low.

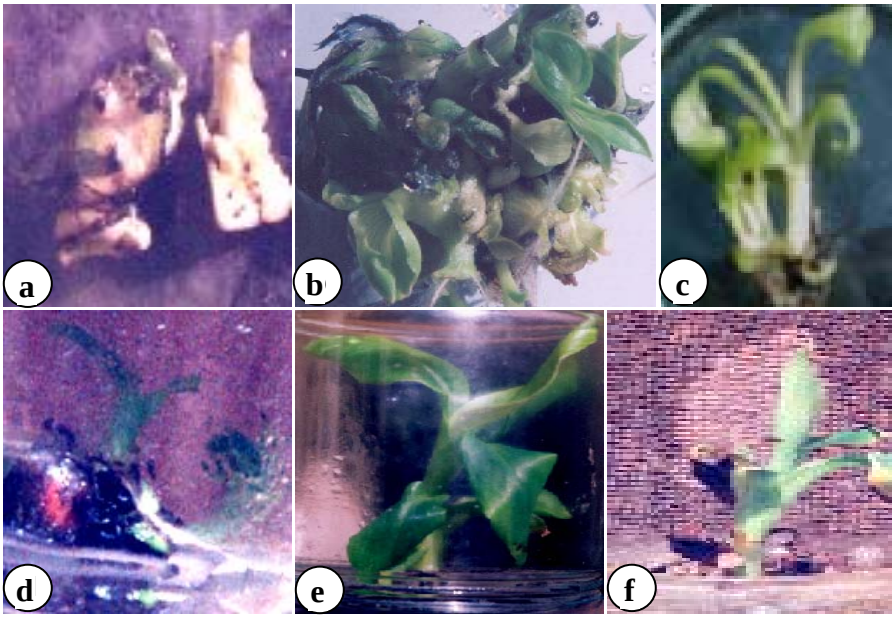

Fig. 2. Different steps for the micro-propagation of banana (*Musa* spp.) cv. Basrai. **a:** Explant proliferation/organgenesis on MS (10.0μM BA; 15.0 μM IAA) medium; **b:** Shoot induction and its multiplication on MS (10.0 μM BA) medium; **c:** Sub-cultured micro-stem on the shoot multiplication medium; **d:** Microstem-cuttings, after 4th sub-culturing on shoot induction medium MS_{2k}; **e:** Root induction in the micro-propagated plantlets on ½ MS_0 basal medium supplemented with IBA (0.10mg/L); **f:** Establishment of banana plantlets in the soil after plant hardening.

After 4th sub-culturing on the shooting medium, well developing plantlets (Fig. 2d; 3c) of about 3 weeks old were excised and cultured on ½ MS basal medium supplemented with 0.1 mg/L IBA for 2-weeks, where 95.58% plantlets were rooted (Table 1; Fig 2e). Rooted plantlets were then transferred to pots (covered with a polythene bags for a few days to prevent wilting) for plant hardening. After 2-weeks, they were established under greenhouse conditions (Fig. 2f).

Discussion

The propagation of the vegetative crops under *In-vivo* is easy but at low rate. The suckers produced by this method are not disease free in comparison to *In-vitro* developed plantlets. Different laboratories are busy to develop any economic procedure for *in-vitro* propagation in different crops. In banana, the most widespread used technique for vegetative propagation is *In-vitro* micro-propagation. Through which, the plantlets can be regenerated by culturing an actively growing part (explant) of the plant on the medium supplemented with specific cytokinins and auxins (Arinaitwe *et al*., 2000; Vuylsteke, 1998; Mendes *et al*., 1999; Wojtania & Gabryszweska, 2001; Ortiz & Vuylsteke, 1994; Madhulatha *et al*., 2006). The shoot regeneration is possible only when explants were cultured on the MS medium supplemented with BA, after organogenesis (*via* IAA and BA; 3-weeks culture). BA is an agent, which can trigger to induce mass proliferation and then shoot induction (Fig. 4b) in banana (cv Basrai) within 2-3 weeks (Daniells, 1997; Jambhale *et al*., 2001; Kadota & Niimi, 2003; Hirimburegama & Gamage, 1997), while

BA was unable to induce explant mass proliferation and shoot induction in an un-organogenised explant (Table 1).

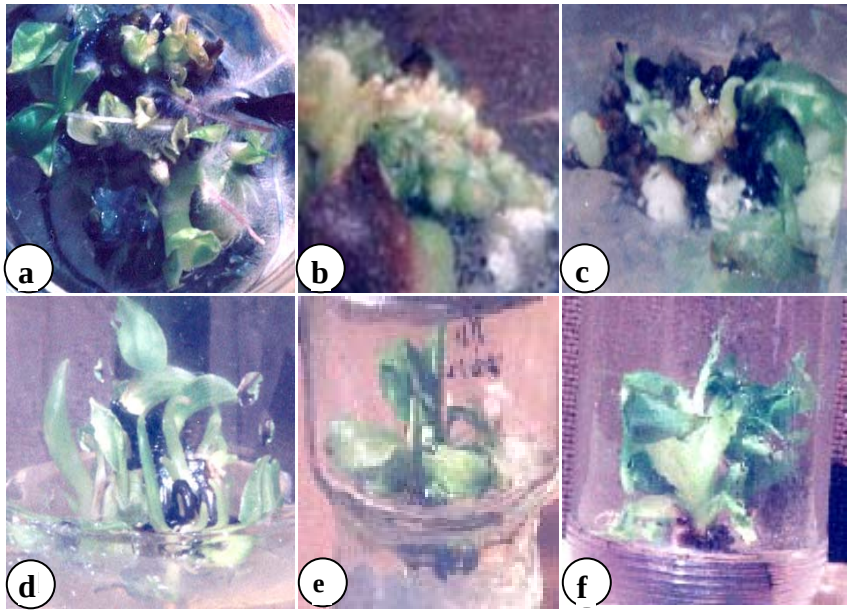

Fig. 3. Different modes of the shoot induction in micro-propagating banana (*Musa* spp.) cv. Basrai plantlets by culturing under different immersion systems. **a:** Shoot induction (3.60 g/L phytagel) after organogenesis on routinely used culture system (S); **b:** Shoot induction (2.0 g/L phytagel) after organogenesis on temporary immersion system (T); **c:** Shoot induction (1.0 g/L phytagel) after organogenesis on permanent immersion system (P); **d:** Micro-propagating plantlets (after 4 weeks) on the temporary immersion system (P) in which the organogenesis was carried out on routinely used culture system (S) for 3 weeks and than to the permanent immersion system (P) for 10 days; **e:** Micro-propagated plantlets growing on rooting medium, which was developed through temporary immersion system; **f:** Micro-propagated plantlets growing on rooting medium, which was developed through routinely used culture system.

So shoot induction and its multiplication is possible in the presence of BA, which is dependent on a critical phenomena "organogenesis", directed by a specific combination of auxins and cytokinins in the medium. Among BA, IAA, NAA and TDZ; a combination of IAA and BA is considered as a good one for organogenesis. The enhanced shoot multiplication rate due to BA in a particular explant is the reflection of IAA and BA, which were used for organogenesis before shoot induction/multiplication (Fig. 4d). In general, higher levels of BA in the medium causes to increase not only the number of shoots per explants, but also abnormal shoot buds also develop, which do not enable itself to develop into a normal plantlets (Arias, 1992; Van den *et al*., 1998; Victor *et al*., 1999).

However, the medium solidification and/or its liquefaction are also effective on the rate of micro-propagation (Be & Debergh, 2006). It causes to develop abnormalities in the multiplying plantlets (Fig. 3a, b, c). Such abnormalities are not easy to release (Vuylsteke & Ortiz, 1996; Matsumoto & Brandao, 2002; Daquinta *et al*., 2000; Murch *et al*., 2004) while developed plantlets are not suitable for further micro-propagation. Normal plantlets can be obtained from abnormal ones by repeating the organogenesis to shoot induction steps again. This is a much laborious process but such developed plants may or may not be fertile in the field.

In banana tissue culture, the goal is to produce a maximum number of shoots with long enough for rooting ability under *In-vitro* conditions. In this aspect, the physical conditions of the medium like as the routinely used tissue culture system, temporary immersion system and permanent immersion system should be used in an interconnected form. Through this optimized protocol (Fig. 1), pathogen free plantlets can be developed within 2-3 months. By using, this optimized protocol, any desired banana (*Musa* spp.) genotype can be micro-propagated within a short time period by making some change in medium composition and/or culture timing.

References

Alvard, D., F. Cote and C. Teisson. 1993. Comparison of methods of liquid medium culture for banana micro-propagation: Effects of temporary immersion of explants. *Plant Cell Tiss. Org. Cult*., 32: 55-60.

Anonymous. 2005. *FAO Production year book*, 2004. Food and Agriculture Organization of the U.N. New York, Vol., 57. ISBN 925002162.

Arias, O. 1992. Commercial micro-propagation of banana. In: *Biotechnology Applications for Banana* and *Plantain Improvement*. Inibap, San Jose, Costa Rica. pp. 139-142.

Arinaitwe, G., P.R. Rubaihayo and M.J.S. Magambo. 2000. Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. *Sci. Hortic*., 86: 13-21.

Be, L.V. and P.C. Debergh. 2006. Potential low cost micro-propagation of pineapple (*Ananas comosus*). *South African J. Bot*., 72: 191-194.

Daniells, J. 1997. Beware the potential hazards of tissue culture. *Infomusa*, 6: 17-18.

Daquinta, M., Y. Lezcano, M. Escalona and R. Santos. 2000. *In vitro* multiplication of the banana FHIA-18 with pactobutrazol and thidiazuron, using different ways of cultivation. *Rev. Bras. Frutic Jaboticabal-SP*, 22: 86-88.

Escalona, M., J.C. Lorenzo, B. Gonzalez, M. Daquinta, J.L. Gonzalez, Y. Desjardins and C.G. Borroto. 1999. Pineapple (*Ananas comosus* L. Merr) micro-propagation in temporary immersion systems. *Plant Cell Rep*., 18:743-748.

Etienne, H. and M. Berthouly. 2002. Temporary immersion in plant micro-propagation. *Plant Cell Tiss. Org. Cult*., 69: 215-231.

Frison, E.A., G. Orjeda and S.L. Sharrock. 1997. PRO*MUSA*: A global programme for *Musa* improvement. In: *Proceedings of a meeting held in Gosier, Guadeloupe*, (Eds.) E.A. Frison and G. Orjeda, G. and S.L. Sharrock. March 5 and 9, 1997 (pp 8–11). International Network for the Improvement of Banana and Plantain, Montpellier, France / The World Bank, Washington, USA

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res*., 50: 151-158.

Georget, R., R. Domergue, N. Ferriere and F.X. Cote. 2000. Morpho-histological study of the different constituents of a banana (*Musa* AAA, cv. Grande naine) embryogenic cell suspension. *Plant Cell Rep*., 19: 748-754.

Hirimburegama, K. and N. Gamage. 1997. Cultivar specificity with respect to in vitro micro-propagation of *Musa* spp. (banana and plantain). *J. Hortic. Sci*., 72: 205-211.

Hwang, S.C., C.L. Chen, J.C. Lin and H.L. Lin. 1984. Cultivation of banana using plantlets from meristem culture. *HortSci*., 19: 231-233.

Jambhale, N.D., S.C. Patil, A.S. Jadhav, S.V. Pawar and B.D. Waghmode. 2001. Effect of number of subcultures on *in vitro* multiplication of four banana clones. *Infomusa*, 10: 38-39.

Kadota, M. and Y. Niimi. 2003. Effects of cytokinin types and their concentrations on shoot proliferation and hyperhydricity *in vitro* pear cultivar shoots. *Plant Cell Tiss. Org. Cult*., 72: 261-265.

Krikorian, A.A. and S.S. Cronauer. 1984. Aseptic culture techniques for banana and plantain improvement. *Econom. Bot*., 38: 322-331.

Ma, S.S. and C.I. Shii. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *J. Chinese Soci. Hort. Sci*., 18: 135-142.

Madhulatha, P., S.I. Kirubakaran and N. Sakthivel. 2006. Effects of carbon sources and auxins on *in-vitro* propagation of banana. *Biologia Plantarum*, 50: 782-784.

Matsumoto, K. and A.K.C. Brandao. 2002. Comparison of temporary and permanent immersion systems for the *in vitro* culture of banana. *Infomusa*, 11: 36-37.

Mendes, B.M.J., S.B. Filippi, C.G.B. Demetrio and A.P.M. Rodriguez. 1999. A statistical approach to study the dynamics of micro-propagation rates, using banana (*Musa* spp.) as an example. *Plant Cell Rep*., 18: 967-971.

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

Murch, S.J., C. Liu, R.M. Romero and P.K. Saxena. 2004. *In vitro* culture and temporary immersion bioreactor production of Crescentia cujete. *Plant Cell Tiss. Org. Cult*., 78: 63-68.

Ortiz, R. and D.R. Vuylsteke. 1994. Genetics of apical dominance in plantain (*Musa* spp. AAB group) and improvement of suckering behavior. *J. Am. Soc. Hortic. Sci*., 119: 1050-1053.

Ray, T., I. Dutta, P. Saha, S. Das and S.C. Roy. 2006. Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell, Tiss. Org. Cult*., 85: 11-21.

Roels, S., M. Escalona, I. Cejas, C. Noceda, R. Rodriguez, M.J. Canal, J. Sandoval and P. Debergh. 2005. Optimization of plantain (*Musa* AAB) micro-propagation by temporary immersion system. *Plant Cell Tiss. Org. Cult*., 82: 57-66.

Roux, N., J. Dolezel, R. Swennen and F.J. Zapata-Arias. 2001. Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* spp. *Plant Cell, Tiss. Org. Cult*., 66: 189-197.

Simmonds, N.W. 1962. *The evolution of the bananas*. Longmans, Green and Co., Ltd. London. pp 170.

Stover, R.H. and N.W. Simmonds. 1987. *Bananas* (3rd edition). Longman, London, UK.

Van-den HI, J. Guns and R. Swennen. 1998. Bacterial contamination in *Musa* shoot tip cultures. *Acta Hort*., 490: 485-492.

Victor, J.M.R., S.J. Murch, R.S. Krishna and P.K. Saxena. 1999. Somatic embryogenesis and organogenesis in peanut: The role of thidiazuron and N6-benzylaminopurine in the induction of plant morphogenesis. *Plant Growth Reg*., 28: 9-15.

Vuylsteke, D. and E. De Langhe. 1985. Feasibility of *In vitro* propagation of bananas and plantains. *Trop. Agr*. (Trinidad), 62: 323-328.

Vuylsteke, D.R. (1998). *Shoot-tip culture for the propagation, conservation* and *distribution of Musa germplasm.* IITA, Ibadan, Nigeria. 82pp.

Vuylsteke, D.R. and R. Ortiz. 1996. Field performance of conventional vs. *in vitro* propagules of plantain (*Musa* spp., AAB group). *HortScience*, 31:862-865.

Wojtania, A. and E. Gabryszweska. 2001. Effect of cytokinins and amino acids on multiplication of *Pelargonium* cultivars. *Acta Soc. Bot. Pol*., 70: 203-207.

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