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Full Length Research Paper

Efficient regeneration of the endangered banana cultivar 'Virupakshi' (AAB) via embryogenic cell suspension from immature male flowers

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Plantlets of the banana cultivar 'Virupakshi' (AAB) were regenerated from somatic embryos derived from embryogenic cells of calli from immature male flower explants. Induction of calli from explants was favored by a relatively moderate concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) (4 mg/L), high concentrations of proline and glutamine (both 300 mg/L) and coconut water. A suspension culture of the callus-derived embryogenic cells in Murashige and Skoog (MS) basal medium (pH 5.3) with 2 mg/L 2,4-D, 1 mg/L indole-3-acetic acid (IAA), 1 mg/L NAA, 45 g/L sucrose and 20 g/L maltose produced synchronously proliferating cells with the potency to be induced into somatic embryos on an 8 g/L agarose-solified SH basal medium (pH 5.7) with recommended vitamins, 1 mg/L IAA, 1 mg/L naphthaleneacetic acid (NAA), 0.2 mg/L 2-ip, 40 g/L sucrose, 20 g/L maltose, 10 g/L dextrose. The young somatic embryos differentiated into regenerable mature somatic embryos on an 8 g/L agarose-solidified MS basal medium (pH 5.7) with 1 mg/L NAA, 100 ml/L coconut water, 30 g/L sucrose, 30 g/L maltose and 100 mg/L glutamine. The mature somatic embryos regenerated into plantlets on a 2.5 g/L gelrite-solidified MS-based medium with 2.5 mg/L 6-benzylaminopurine (BAP), 1 mg/L gibberellic acid (GA_3), 100 mg/L L-glutamine, 30 g/L sucrose and 2.5 mg thidiazuron (TDZ). The total duration from explant stage, for the development of plantlets of 10 to 15 cm height, which could withstand hardening process, was 16 months. The plantlets were morphologically normal, suggesting normal development without somaclonal variation. The present regeneration protocol for 'Virupakshi' has great potential for preserving the endangered germplasm by micropropagation and its improvement by transgenic technology against the deadly Banana bunchy top virus and other economically important diseases.

Key words: Hill banana, *in vitro* micropropagation, male flower bud explants, somatic embryogenesis, Virupakshi.

INTRODUCTION

The banana cultivar 'Virupakshi' (*Musa* AAB, Pome subgroup) is one of the few elite hill bananas that are cultivated exclusively in the Lower Pulneys, Sirumalai and Kolli Hills of the Western Ghats of India at an altitude of 2000 to 5000 msl. The cultivar is highly desired for its unique flavor, taste and religious significance. The bunch weight ranges from 10 to 15 kg and the fruits have a relatively long shelf life and low water content.'Virupakshi' is highly susceptible to the banana bunchy top disease caused by banana bunchy top virus (BBTV) (family *Nanoviridae*). Consequently, the area under 'Virupakshi' cultivation has decreased from an estimated 18,000 ha in the 1970s to around 2,000 ha to date (Elayabalan et al., 2012) BBTV is transmitted by the aphid *Pentalonia*

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nigronervosa (Hu et al., 1996). The year-round presence of the vector maintains a source of inoculum that infects new plantations of the cultivar 'Virupakshi'continually. The lack of clean BBTV-free planting material is currently a serious constraint for the continued production of this cultivar. The constant threat of the BBTV epidemic has discouraged farmers from opting for this cultivar. There is danger that the cultivar will be lost and even become extinct if appropriate measures are not taken.

Genetic improvement of bananas by conventional breeding is seriously limited by factors such as lack of genetic variability, ploidy variations and low female and male fertility (Vuylsteke and Swennen, 1992; Pillay et al., 2012). One approach to overcoming these problems is to develop transgenic bananas, and in the case of 'Virupakshi' with resistance to BBTV. Embryogenic cell suspensions of a banana cultivar provide ideal material for developing transgenic banana. Banana regeneration has been achieved from various explants, including suckers, shoot tips (Panis and Swennen, 1993), zygotic embryos (Escalant and Tession, 1989), immature male flowers (Cote et al., 1996; Navarro et al., 1997; Grapin et al., 1998; Sagi et al., 1994; Ganapathi et al., 1999; Becker et al., 1998; Assani et al., 2001; Jalil et al., 2003; Mahadev et al., 2011), multiple meristem (Strosse et al., 2003; Sholi et al., 2009) and anthers (Assani et al., 2003). Among the various explants used, immature male and female flowers appear to be the most responsive starting materials for embryogenic callus induction, embryogenic cell suspension, and somatic embryo induction and regeneration. *In-vitro* regeneration has been reported for several cultivars of banana, with a regeneration period ranging from 10 to 18 months (Cote et al., 1996; Becker et al., 1998; Ganapathi et al., 2001).

The objective of the current research was to optimize an *in vitro* propagation technique for 'Virupakshi' through embryogenic cell suspension culture. An embryogenic cell suspension culture will provide valuable materials for genetic modification of 'Virupakshi'cultivar for incorporating genes for host plant resistance to pathogens, pests and desirable horticultural traits.

MATERIALS AND METHODS

Plant material

Male inflorescences from banana bunchy top virus free 'Virupakshi' plants were obtained from an orchard in the Lower Pulneys Hills, located at 2000 to 5000 feet msl in the Western Ghats of Tamil Nadu. The immature male flower buds occupying positions 1 to 15 (1 being the position closest to the meristematic dome) (Figure 1A) were extricated aseptically as described by Ram et al. (1967) and Ma (1988).

Explant preparation and embryogenic callus induction

The extricated buds were surface-sterilized for 1 min in 70% ethanol and trimmed to a size of ~0.8 x 2 cm in length. The explants were placed individually (Figure 1B) on MS-based embryogenic callus induction (ECI) medium-1, 2, 3, 4 and 5 (Table 1) in 100×120 mm Petri dishes. The plates were incubated in the dark at 18°C. Sub-culturing was done once every 10 days, until embryogenic calli developed.

Initiation of embryogenic cell suspension culture

Six-month old embryogenic calli (Figure 1C) were used to initiate an embryogenic cell suspension culture of 'Virupakshi'. About 100 mg embryogenic calli was transferred to 100 ml conical flasks (Figure 1D) with 10 ml liquid medium containing Murashige and Skoog (1962) (MS)-based embryogenic cell suspension (ECS) medium (pH 5.3) with 2 mg/L 2,4-dichlorophenoxyacetic acid (2, 4-D), 1 mg/L indole-3-acetic acid (IAA), 1 mg/L naphthalene acetic acid (NAA), 45 g/L sucrose and 20 g/L maltose. The ECS culture was incubated at 18 to 25±1°C under constant shaking (100 rpm) and maintained under a light and dark cycle of 16 and 8 h, respectively, with weekly replacement of the spent medium by fresh medium. At each sub-culturing stage, an aliquot of the suspension culture was observed under the light microscope to ascertain the presence of embryogenic cells.

Induction of somatic embryos from embryogenic cells

1 ml of regenerable embryogenic cell suspension (Figure 1E) was plated on three different semisolid somatic embryo induction (SEIM) media, that is, MS-based SEI medium-1 and SH-based SEI media -2 and -3 (Schenk and Hildebrandt, 1972) (Table 1). The cultures were incubated at 26°C in a growth chamber with light/dark cycle of 16/8 h, respectively, until somatic embryos were observed.

Maturation of somatic embryos and regeneration of plantlets

The somatic embryos were incubated for two months on 1x MS based somatic embryo maturation media-(SEMM)-1, 2 and 3 (Table 1). After two months, mature somatic embryos (Figure 1F) were transferred to 0.5x MS based regeneration medium (RM) 1, 2 and 3 (Table 1). The regenerated plantlets (~10 to 15 cm in height) with two to three leaves were removed from the culture medium, washed to remove the gelrite and then transplanted into 90-well trays containing sterilized soil mixture (sand: clay soil: coir pith = 1:1:1) and irrigated with Hoagland's medium (Hoagland, 1950). The trays were covered with clear plastic bags to conserve moisture. After three to four weeks of primary hardening, each plant was transplanted into a pot (9 × 9 × 36 cm) and maintained in a green house till the plant reached a height of 20 to 25 cm. The hardened plants were transferred to the field.

Data analysis

Data analysis was done by AGRES statistical package. Mean values were separated by Duncan's multiple range test (DMRT) at 5% probability level (Duncan, 1955). In case of percentage, arcsin $(p\%/100)^{½}$ transformation of the variable was performed before analysis, and were converted back to percentages for presentation.

RESULTS AND DISCUSSION

Embryogenic callus induction

Among the different media tested for embryogenic callus

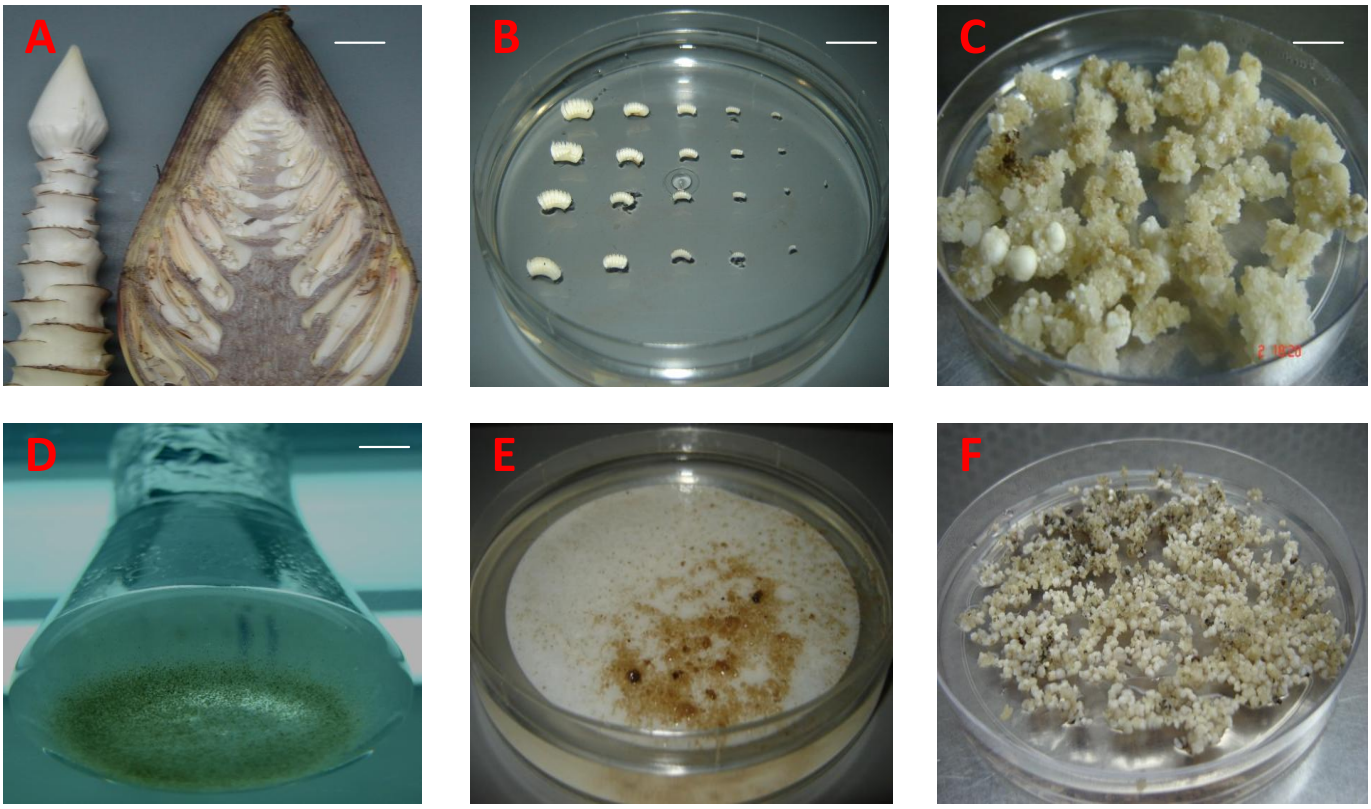

Figure 1. (A) Male bud 1 to 15th position. **(B)** Inoculated immature male flower. **(C)** Induction of embryogenic calli. **(D)** Initiation of embryogenic cell suspension; bar = 2.5 mm. **(E)** Induction of somatic embryo. **(F)** Maturation of somatic embryo. Bars A, B, C, E and F = 20 mm.

induction of cultivar 'Virupakshi' (Table 1), the highest frequency of callus formation (57.78) occurred in the MS-based ECIM3 (Table 2). The composition of ECIM3 differed from those of the other media by having a moderate concentration of 2,4-D (that is, 4 mg/L, while the 2,4-D levels in other ECI media varied between 2 to 6 mg/L); a high concentration of proline (that is, 300 mg/L, while the others had only 150 mg/L); glutamine (300 mg/L), which was absent in other ECI media, and 1.5 g/L phytagel. The other ECI media either completely lacked phytagel or had concentrations of 3 g/L. Although, both ECIM2 and ECIM3 contained coconut water, they differed in the concentrations of growth factors and amino acids (Table 1). A higher frequency of callus induction in media containing 2,4-D (4 mg/l) was also reported by Cote et al. (1996), Grapin et al. (2000), Assani et al. (2001) and Ganapathi et al. (2001) in other banana cultivars.

Callus tissue appeared as a protrusion from the basal portion of the explant. These primordial protrusions grew into larger translucent calli, which eventually developed into masses of yellowish-white embryogenic calli (Figure 1C). Callus growth was often associated with exudation of phenolic compounds, as evidenced by browning of calli and the brown exudation in the surrounding medium. One of the problems encountered in plant tissue culture is the rapid browning, hyperhydricity and eventual decay of calli due to exudation of phenolics into the media. Similar problems were encountered in this study. Varying degrees of exudation of phenolic compounds were observed with the different media. The calli grown on media with coconut water and L-proline produced little or decreased amounts of phenolics compared to the other media. Moreover, the medium supplemented with coconut water and L-proline supported better growth of calli and differentiation of calli into somatic pre-embryos.

It is known that phenolic compounds from calli inhibit the growth and differentiation of callus tissue (Strosse et al., 2003). Phenolic compounds in calli result from the stress caused by cellular competition for nutrients and space, and local accumulation of catabolites around the calli. The degree of browning of calli appears to vary depending on the composition of media. Moreover, the degree of exudation of phenolics and resultant browning may also be affected by the growth promoters and other factors used and their concentrations. Novak et al. (1989) and Strosse et al. (2003) reported browning of calli prior to pre-embryo stage in media containing high concentrations of 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and picloram.

Regular sub-culturing, once in a week, reduced

Table 1. Media composition used for regeneration protocol for hill banana cv.Virupakshi (AAB).

Medium composition	Media														
	ECIM1	ECIM2	ECIM3	ECIM4	ECIM5	ECSM	SEIM1	SEIM2	SEIM3	SEMM1	SEMM2	SEMM3	RM1	RM2	RM3
MS components	1x	1x	1x	1x	1x	1x	-	-	1x	1x	1x	1x	0.5x	0.5x	0.5x
SH components	-	-	-	-	-	-	1x	1x	-	-	-	-	-	-	-
2,4-D (mg)	6	2	4	4	-	2	-	-	-	-	-	-	-	-	-
Dicamba (mg)	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
IAA (mg)	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-
NAA (mg)	1	1	1	1	1	1	1	1	1	-	-	1	-	-	-
2ip (mg)	-	-	-	-	-	-	0.2	0.2	0.2	-	-	-	-	-	-
BAP (mg)	-	-	-	-	-	-	-	-	-	-	-	-	2	2.5	2
GA_3 (mg)	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
TDZ (mg)	-	-	-	-	-	-	-	-	-	-	-	-	-	2.5	-
MS vitamins	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
MAs vitamins	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
CW (ml)	-	100	100	-	-	-	-	-	-	-	-	100	-	-	-
L-Proline(mg)	150	150	300	150	150	-	-	-	-	-	-	-	-	-	-
L-Glutamine(mg)	-	-	150	-	-	100	100	100	100	-	-	100	100	100	100
Biotin (mg)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-
Sucrose (g)	30	30	30	30	30	45	45	40	40	-	30	30	30	30	30
Maltose (g)	-	-	-	-	-	20	-	-	20	30	-	30	-	-	-
Dextrose (g)	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-
Phytagel (g)	-	3	1.5	3	3	-	3	3	-	-	-	-	-	-	-
Agarose (g)	8	-	-	-	-	-	-	-	8	4	2.5	8	-	-	-
Gelrite (g)	-	-	-	-	-	-	-	-	-	-	-	-	2.5	2.5	2.5
pH	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7

ECIM, Embryogenic callus induction medium; ECSM, embryogenic cell suspension medium; SEIM, somatic embryo induction medium; SEMM, somatic embryo maturation medium; RM, regeneration medium; CW, coconut water.

exudation of phenolic compounds, which otherwise would have affected embryogenic calli development. Exudation of phenolic compounds and browning were at the lowest in ECIM3, which contained coconut water and a lower percentage of phytagel (1.5 g/L). After a period of approximately five months in the callus induction medium, embryogenic calli with pro-embryos were observed under a stereo microscope.

Initiation of embryogenic cell suspension and induction of somatic embryos

Portions (0.1 g) of embryogenic calli with pro-embryos were used to produce cell suspensions in embryogenic cell suspension (ECS) medium [that is, MS basal medium (pH 5.3) supplemented with 2 mg/L 2,4-D, 1 mg/L IAA, 1 mg/L NAA, 45 g/L sucrose and 20 g/L maltose]. The cells attained uniform maturation within three months (Figure 1D). The embryogenic cells proliferated rapidly, probably due to the high concentration of carbon (45 g/l sucrose + 20 g/l maltose) in the medium. Dense starch granules were observed in the cytoplasm of the embryogenic cells under a compound microscope (40x). It was possible to distinguish the spherical embryogenic cells with dense starch granules from the elongated or

Table 2. Embryogenic callus induction from male buds of hill banana.

Media	Number* of EC (%±SE)
ECIM1	46.67±1.1b
ECIM2	53.33±0.5ab
ECIM3	57.78±0.2a
ECIM4	46.67±1.1b
ECIM5	40.24±1.5c

Three replications maintained with15 explants per replicate. Data was taken six months after embryogenic calli induction. In a column, means followed by a common value are not significantly different at the 5% level by DMRT.

Table 3. Somatic embryo induction, maturation and regeneration.

Number of EC used (1 EC/replication)	Media	Number of SE induced/ml ECS*	Media	Number of SE matured ** (%±SE)	Media	Number of SE germinated*** (%±SE)
3	SEIM1	11.3×10^3	SEMM1 (3000)	85.83 ± 47^b (2109)	RM1 (1500)	56.66 ± 12^b (849)
3	SEIM2	10.3×10^3	SEMM2 (3000)	55.33 ± 80^c (1629)	RM2 (1500)	92.53 ± 12^a (1387)
3	SEIM3	14.4×10^3	SEMM3 (3000)	90.33 ± 26^a (2574)	RM3 (1500)	38.3 ± 73^c (1095)

In a column, means followed by a common value are not significantly different at the 5% level by DMRT. *Each treatment had three replications and observations were taken after three months of somatic embryo induction. **Each treatment had three replications with 1000 embryos per replication. Observations were taken after three months of somatic embryo maturation. ***Each treatment had three replication with 500 embryos per replication. Observations were taken after three months of somatic embryo germination.

irregularly shaped non-embryogenic cells lacking starch granules. This study shows that SEIM3 (pH 5.7) that was composed of a combination of three carbon sources (40 g/L sucrose,20 g/L maltose, and 10 g/L dextrose) in agarose (8 g/L) produced the greatest number of embryos. An estimated 14.4×10^3 embryos were obtained from each milliliter of SEIM3 (Figure 1E).

Somatic embryo maturation and plantlet development

Somatic embryo maturation occurred best (Table 3) in SEMM3 (that is, 8 g/L agarose-solidified MS basal medium (pH 5. 7) with 1 mg/L NAA, MS vitamins, glutamine 100 mg/L, coconut water 100 ml/L, 30 g/L sucrose and 30 g/L maltose). The composition of SEMM3 differed from those of SEMM1 and SEMM2 by containing NAA, coconut water and glutamine. In addition, SEMM3 contained both sucrose (30 g/L) and maltose (30 g/L) as carbon sources, while the other SEMMs contained only one carbon source. Agarose was used as the solidifying media in SEMM3 at 8 g/L, while the other SEMMs contained agarose at 2.5 or 4 g/L.

The highest regeneration efficiency (93%) was obtained in regeneration medium-2 [that is, a 2.5 g/L gelrite-solidified MS basal medium (pH 5. 7) supplemented with 2.5 mg/L 6-benzylaminopurine (BAP), 1 mg/L gibberellic acid (GA_3), 2.5 mg/L thidiazuron (TDZ), MS vitamins, 100 mg/L glutamine and 30 g/L sucrose]. The other media did not contain the diphenylurea-derivative cytokinin TDZ.

Thidiazuron has not been widely used in the micropropagation of *Musa*. Most protocols involving *Musa* micropropagation have used adenine-derived cytokinins such as BAP, 2-isopentenyladenine (2-2ip) and zeatin (Talengera et al., 1994; Crouch et al., 1998). Thidiazuron has been shown to induce habituation (Huetteman and Preece, 1993; Sujatha and Reddy, 1998) and (relative) cytokinin-independence (Neuman et al., 1993) in some species. This desirable biological property makes the use of TDZ in micropropagation cost effective. The habituation effect of TDZ was shown in several banana cultivars when banana shootlets were able to proliferate on hormone-free media (Makara et al., 2010). In the present investigation, the high regeneration efficiency (93%) obtained with medium-2 is probably attributable to the effect of TDZ, since the other regeneration media, which only contained adenine-derivative cytokinins, were less productive.

The somatic embryos differentiated into translucent spherical to torpedo shaped structures, representing different embryonic stages in SEM-2. These embryos, some of which turned green due to genesis of chloroplasts, produced adventitious buds that differen-

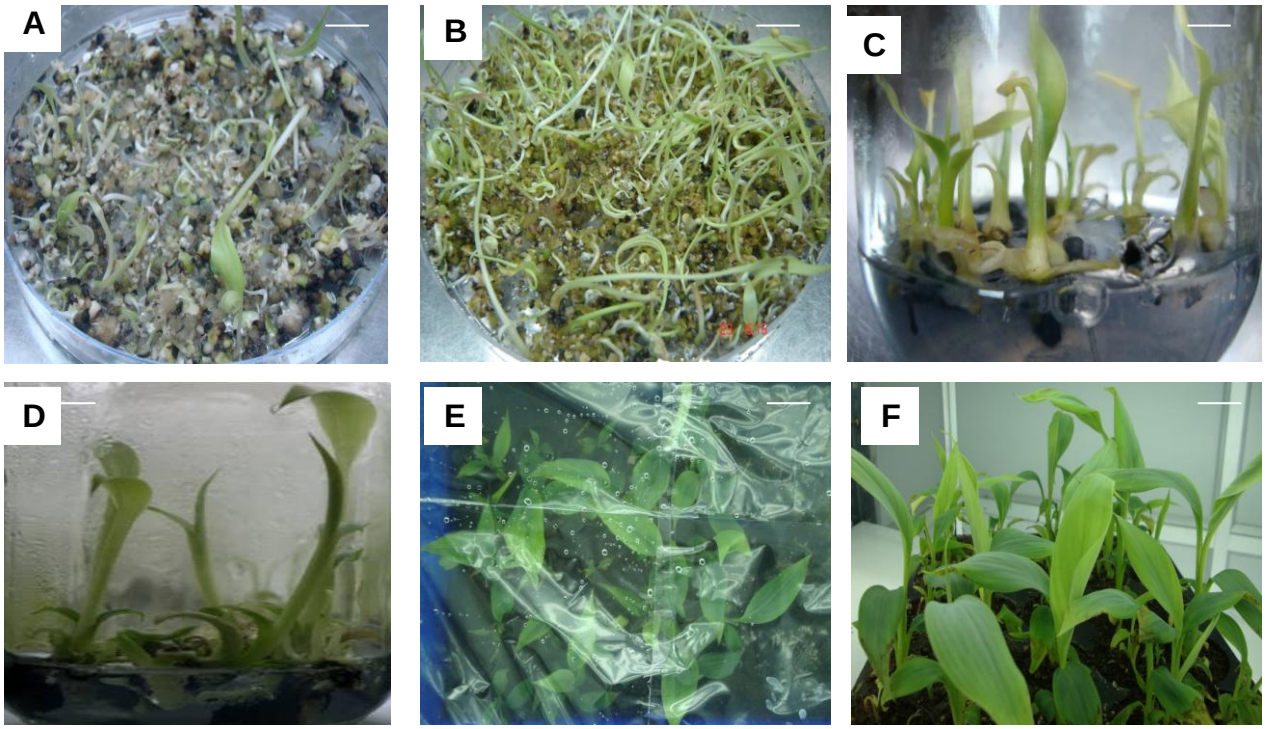

Figure 2. (A) Germination of somatic embryo. **(B)** Shoot development. **(C)** Shoot and root development. **(D)** Complete plantlet development. **(E)** Primary hardening in 90 well trays covered with poly bag. **(F)** Plants ready for secondary hardening. Bars A and B = 20 mm.

tiated into green shootlets (Figures 2A, B and C). This study shows that an MS-based maturation medium consisting of coconut water coupled with a higher concentration of carbon sources and with agarose instead of phytagel was most effective for uniform maturation of embryos. Perhaps, the cytokinins present in coconut water boosted somatic embryo maturation.It is known that when added to a medium containing auxin, coconut water can induce plant cells to divide and grow rapidly (Molnar et al., 2011). Coconut milk was also shown to have produced the highest number and length of both shoots and roots in banana (Beshir et al., 2012).

Growth and development of *in vitro* developed plants

The somatic embryos in regeneration medium-2 differentiated and produced green shoot primordia that grew into shootlets of ~1 to 2 cm in height within 45 days (Figures 2A and B). When subcultured on the same medium for a further 45 days, the shootlets reached a height of ~ 10 cm (Figures 2C and D). During this period, the root primordia gave rise to roots. These plantlets were grown under moisture-preserving transparent polyethylene bags for a week (Figure 2E) and for a further two weeks under direct green-house conditions when they reached a height of ~15 cm. The plantlets produced juvenile lanceolate leaves characteristic of vegetatively developing ‘Virupakshi’ suckers (Figure 2F). The plantlets were then transplanted into individual pots (9 × 9 × 36 cm) containing standard pot mixture and irrigated with 0.5x Hoagland’s solution. The plantlets reached a height of ~25 cm within a period of three weeks and were transplanted to the field. The field-grown plants reached a height of ~1.8 m after eight months and produced 10 to 12 leaves and two to three suckers. The micropropagated plants were morphologically normal and similar to those propagated from suckers. No evidence of any somaclonal variation was observed at this stage of plant growth.

Conclusion

This study produced optimized conditions for embryogenic callus induction, establishment of cell suspension culture, somatic embryo induction and development and regeneration for micropropagation of the endangered banana cultivar ‘Virupakshi’. The combined use of three different cytokinins, that is, the diphenyl urea-derived cytokinin TDZ (2.5 mg/l) and adenine-derived cytokinins BAP (2.5 mg/l) and GA_3 (1 mg/l) appeared to increase the regeneration efficiency of somatic embryos in ‘Virupakshi’. A correlation was found between cytokinin levels of the medium and shoot proliferation and subsequent development of plants.

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Abbreviations

BBTD, Banana bunchy top disease; **BBTV,** banana bunchy top virus; **ECS,** embryogenic cell suspension; **DMRT,** Duncan's multiple range tests; **2ip,** 2-isopentenyladenine; **EC,** embryogenic callus; **SE,** somatic embryo.

REFERENCES

Assani A, Bakry F, Kerbellec F, Haicour R, Wenzel G, Fehr W (2003). Production of haploids from anther culture of banana (*Musa balbisiana* (BB)). Plant Cell Rep. 21:511-516.

Assani A, Haicour R, Wenzel G, Cote F, Bakry F, Wehr BF, Ducreux G, Aguillar ME, Grapin A (2001). Plant regeneration from protoplasts of dessert banana cv. Grande Nain (*Musa* spp. Cavendish sub-group AAA) via somatic embryogenesis. Plant Cell Rep. 2:482-488.

Becker DK, Dugdale B, Smith MK, Harding RM, Dale JL (1998). Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. Grand Nain via microprojectile bombardment. Plant Cell Rep. 19:229-234.

Beshir I, Sharbasy SE, Safwat G, Diab A (2012). The effect of some natural material in the development of shoot and root of banana (*Musa* spp.) using tissue culture technology. New York Sci. J. 5:132-138.

Cote FX, Domergue R, Monmarson S, Schwendiman J, Teisson C, Escalant JV (1996). Embryogenic cell suspensions from the male flower of Musa AAA cv. Grand naine. Physiol. Plant. 97:285-290.

Crouch JH, Vuylsteke D, Oritz R (1998). Perspectives of application biotechnology to assist genetic enhancement of plantain and banana (*Musa* spp.) Plant Biotechnol. 1:1-12.

Duncan DB **(**1955). Multiple range and multiple F test. Biometrics 11:1-42

Escalant JV, Teisson C (1989). Somatic embryogenesis and plants from immature zygotic embryos of species *Musa acuminata* and *Musa balbisiana*. Plant Cell Rep. 7: 665-668.

Ganapathi TR, Higgs NS, Balint-Kurti PJ, Arntzen CJ, May GD (2001) Agrobacterium– mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB). Plant Cell Rep. 20: 157-162.

Ganapathi TR, Suprasanna P, Bapat VA, Kulkarni VM, Rao PS (1999) Somatic embryogenesis and plant regeneration from male flower buds in banana. Curr. Sci. 76:1228-1231.

Grapin A, Ortiz JL, Babeau R, Monmarson J, Escalant S, Teisson JV, Cote F (1998). Establishment of embryogenic callus and initiation and regeneration of embryogenic cell suspensions from female and male immature flowers of *Musa*. InfoMusa 7:13-15.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. Circular 347. California Agricultural Experimental Station, University of California, Berkely, California, USA. 32 pp.

Huetteman CA, Preece JE (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss. Org. Cult. 33:105-119.

Hu JS, Wang M, Sether D, Xie W, Leonhardt KW (1996). Use of polymerase chain reaction (PCR) to study transmission of Banana bunchy top virus by the banana aphid (*Pentalonia nigronervosa*). Ann. Appl. Biol. 128:55-64.

Jalil M, Khalid N, Othman RY (2003). Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). Plant Cell Tiss. Organ Cult. 75:209-214.

Ma SS (1988). Somatic embryogenesis and plant regeneration from cell suspension culture of banana. In: Proceedings of Symposium on Tissue culture of Horticultural Crops, Taipei, Taiwan, 8-9. pp. 181-188.

Mahadev SR, Kathithachalam A, Marimuthu M (2011). An efficient protocol for large-scale plantlet production from male floral meristems of *Musa* spp. Cultivars 'Virupakshi' and 'Sirumalai'. *In vitro* Cell. Dev. Biol. Plant. 47:611–617.

Makara AM, Rubaihayo PR, Magambo MJS (2010). Carry-over effect of Thidiazuron on banana *in vitro* proliferation at different culture cycles and light incubation conditions. Afr. J. Biotechnol. 9:3079-3085.

Molnar Z, Virag E, Ordog V (2011). Natural substances in tissue culture media of higher plants. Acta Biologica Szegediensis 55:123-127.

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

Navarro C, Escobedo RM, Mayo A (1997). *In vitro* plant regeneration from embryogenic cultures of a diploid and triploid Cavendish banana. Plant Cell Tiss. Organ Cult. 51:17-25.

Neuman MC, Preece JE, Van Sambeek JW,Gaffney GR (1993). Somatic embryogenesis andcallus production from cotyledon explants of Eastern black walnut. Plant Cell Tiss. Organ Cult. 32:9-18

Novak FJ, Afza R, Duren M, Perea-Dallos M, Confer BV, Xiolang T (1989). Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.). Biotechnology 46:125-135.

Panis B, Swennen R (1993). Embryogenic *Musa* plant cell cultures: current and future applications. InfoMusa 2:3-6.

Pillay M, Tenkouano A, Ortiz R (2012). Introduction. In: Pillay M, Ude G, Kole C(eds) Genetics, Genomics and Breeding of Bananas, Science Publishers, Enfield, NH, pp. 1-33.

Ram HYM, Ram M, Steward FC (1962). Growth and development of the banana plant. Ann. Bot. 26:657-673.

Sagi L, Remy S, Panis B, Swennen R, Volckaert G (1994). Transient gene expression in electroporated banana (*Musa* spp. cv. 'Bluggoe', ABB group) protoplast isolated from regenerable embryogenic cell suspensions. Plant Cell Rep. 13(5):262-266.

Schenk RU, Hildebrandt AC (1972). Medium and techniques for induction and growth of monocotyledonous and dicotylodonous plant cell cultures. Can. J. Bot. 50:199-204.

Sholi NJY, Chaurasia, A, Agrawal A, Sarin NB (2009). ABA enhances plant regeneration of somaticembryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* sp.). Plant Cell Tiss. Organ Cult. 99:133-140.

Strosse H, Domergue R, Panis B, Escalant JV, Côte F (2003). Banana and plantain embryogenic cell suspensions. In Vézina A, Picq C (ed). INIBAP TechnicalGuidelines 8. The International Network for the Improvement of Banana and Plantain, Montpellier, France.

Sujatha M, Reedy TP (1998). Differential cytokinin effects on the stimulation of in vitro shoot proliferation from meristematic explants of castor (*Ricinus communis* L.). Plant Cell Rep. 17: 561-566.

Talengera D, Magambo MJS, Rubaihayo PR (1994). Testing for a suitable medium for propagation of East African Highland bananas. Afr. Crop. Sci. J. 2:7-21.

Vuylsteke D, Swennen R (1992). Biotechnological approaches to plantain and banana improvement at IITA. In: Thottapilly G, Monti L, Mohan Raj DR, Moore AW (eds)Biotechnology:Enhancing Research on Tropical Crops in Africa. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, pp. 143-150.