SOMATIC EMBRYOGENESIS FROM IMMATURE MALE FLOWERS AND MOLECULAR ANALYSIS OF REGENERATED PLANTS IN BANANA 'LAL KELA' (AAA)

Sidha Meenakshi[1], Bansi Narayanrao Shinde[1]
and Penna Suprasanna[2]

[1]Department of Horticulture, Marathwada Agricultural University, Parbhani, INDIA
[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre
Trombay, Mumbai, 400085, INDIA
e-mail: penna888@yahoo.com; meenakshisidha@gmail.com

A B S T R A C T

Development of embryogenic cultures having high regeneration efficiency from important, commercial varieties of banana is a prerequisite for genetic manipulation and for *in vitro* propagation. In the present study, we have studied the induction of somatic embryogenesis from young immature male inflorescences of the banana cultivar Lal Kela (red banana) on M2 medium (Ma et al., 1991) supplemented with 2,4-D. Cell suspension cultures initiated from this callus exhibited embryogenic stages. Somatic embryos developed into plantlets on ½ strength MS basal medium with 100 mg l^{-1} ME + 0.1% AC and 0.2% gelrite. Plants regenerated through somatic embryogenesis showed minor variation when assessed by randomly amplified polymorphic DNA (RAPD) and sequence characterized amplified regions (SCAR) markers.

Key words: somatic embryogenesis, banana, *Musa*, genetic fidelity, plant regeneration

INTRODUCTION

Banana (*Musa* sp. L.) is the fourth most important agricultural commodity after rice, wheat and corn, and is produced in the tropical and subtropical regions. Banana is grown in more than 130 countries throughout the world on an area 8.25 million ha with a production of 97.38 million tons (Singh, 2007). India is the largest producer of banana in the world contributing 19.71 percent to the global production of banana (Singh, 2007). Banana production is hampered by pests and bacterial and fungal diseases (Stover, 1980). Improvement by using classical breeding

techniques has been difficult and time consuming due to the poly-ploidy, sterility, and long generation period of most edible cultivars.

Musa acuminata (AAA) 'Lal Kela' is commonly known as red banana. In India, it is the most relished and highly prized variety, being prominent in the Indian states of Maharashtra, Tamil Nadu and Kerala (Menon, 2000). The scarce distribution of the 'Lal Kela' is due to several factors: the non-availability of good quality, disease-free planting material, well maintained hygienic field practices and the prevalent attack of bacterial and viral infections. Developing *in vitro* cultures having high frequency of plant regeneration from commercial important varieties of banana is a prerequisite for realizing the potential for large scale production of planting material and cellular and molecular approaches for crop improvement (Smith et al., 2005). Among the different explants (proliferating meristem sections, rhizomes, leaf bases, immature zygotic embryos and young male flowers), immature male inflorescences and proliferating meristem sections (scalps) have mostly been used to initiate embryogenic cultures of several banana and plantain cultivars (Escalant et al., 1994; Côte et al., 1996; Navarro et al., 1997; Becker et al., 2000; Ganapathi et al., 1999; Suprasanna et al., 2002; Kulkarni et al., 2006). Embryogenic cell suspensions (ECS) have become the choice target system for genetic manipulation using different biotechnological tools (Ganapathi et al., 2002).

In this regard, studies on the assessment of the embryogenic potential of commercial cultivars have great relevance. The present study shows results from: the induction of somatic embryogenesis, plant regeneration in a commercially important banana cultivar ('Lal Kela', AAA), and assessing variation among the regenerated plants using molecular markers.

MATERIAL AND METHODS

Plant material, sterilization and culture media

Male flower buds of the 'Lal Kela' (AAA) banana were collected from the field-grown plants, 1 to 10 weeks after flowering. The male buds were shortened to 6-8 cm in height by removing the enveloping bracts outside the laminar hood. These explants were surface sterilized in 70% ethanol for about 5 minutes and rinsed three to five times in sterile water. The male buds were reduced to 1.5 cm in length for culture. Immature male flower clusters from position 0-15 were removed and cultured on MS basal medium (Murashige and Skoog, 1962) with different plant growth regulators. Callus induction medium (designated as M1 medium) was supplemented with 18.10 μM 2,4-dichlorophenoxy acetic acid (2,4-D), 5.71 μM indole-3-acetic acid (IAA), 5.37 μM α-naphthalene acetic acid (NAA), d-biotin (1 mg l^{-1}), sucrose (3%) and Gelrite (0.2%) as a gelling agent. For the proliferation and maintenance of the callus, MS medium supplemented with 4.52 μM 2,4-D, 1 mg l^{-1}biotin, 100 mg l^{-1} malt extract,

16

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

100 mg l^{-1} glutamine, 4.5% sucrose and 0.2% gelrite (designated as M2 medium) (Ma, 1991) was used. Data on the frequency of callus induction and embryogenic callus formation were recorded from the flower buds cultured on the respective medium.

Somatic embryo development and plant regeneration

For the development of the somatic embryos, medium (designated as M3 medium) based on Schenk and Hildebrandt (1972) was supplemented with MS vitamins, glutamine (100 mg l^{-1}), malt extract (100 mg l^{-1}), picloram (1 mg l^{-1}), 4.5% sucrose and 0.2% gelrite. The embryo development was achieved by plating 0.1 ml packed cell volume (PCV) on the above medium. For somatic embryo to plant conversion, MS medium supplemented with benzylaminopurine (BAP, 2.22 µM), IAA (1.14 µM), sucrose (3%) and gelrite (0.2%) was used, while for further plant regeneration, MS medium supplemented with BAP (8.9 µM), sucrose (3%) and gelrite (0.2%) was used. Different levels of BAP (0.02-20 mg/l) were also used to test the effect of BAP on plant regeneration. For each treatment, 50 embryos were tested. For the root induction of plants with good shoot system, MS medium was supplemented with NAA (5.37 µM), sucrose (3%) and gelrite (0.2%). The experiments were conducted in a completely randomized design (CRD). The data were elaborated with the analysis of variance (ANOVA) and the significance of different treatments, as suggested by Panse and Sukhatme (1985).

Acclimatization

The well-developed tender rooted plantlets were washed under running tap water. The rooted plantlets were separated and transferred into a poly bag containing fine sterilized sand, sterilized soil and a farmyard manure (1:1:1) mixture. These plantlets were maintained at a temperature of 28 ℃ and at a relative humidity greater than 90% for the first 15 days. After 15 days, plantlets were kept under diffused normal light. The plants were maintained for 50-60 days for further hardening and growth.

Induction and growth of cell suspension cultures

Cell suspension cultures were initiated by placing embryogenic callus in 100 or 250 ml Erlenmeyer flasks dispensed with 25 or 70 ml liquid M2 medium. To check for growth and proliferation capacity, 1 ml PCV (packed cell volume) was taken from growing suspension culture and grown in fresh M2 liquid medium. The growth was monitored over a period of 1-12 days and on alternate days; the growth was checked by harvesting 1 ml of cells for weighing on sterile glass fibre filters and reculturing them on fresh medium. The number and size of the embryogenic clumps were also measured at 100 x magnification under a microscope. The growth rate over initial, percent growth rate over average, and the compound growth rate was calculated as per the equation of linear regression and a sigmoid curve. The growth of the cell cultures was monitored at one day intervals and a statistically linear regression curve was made

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

17

to study the unit growth rate per day, growth rate over initial, percent growth rate over average and compound growth rate i.e., increase per day and the growth curve was drawn up from measurements of packed cell volume. The number of embryogenic clusters and their size was also recorded according the equation: $\hat{y} = 0.0309 + 0.0384$ 'x' days.

Molecular analysis of regenerated plants of 'Lal Kela' using RAPD and SCAR markers DNA extraction and polymerase chain reaction (PCR) analysis

Total DNA was isolated from the fresh young leaves of the somatic embryo derived regenerants according to the method described by Stewart and Via (1993) with some modifications. The optimum reaction conditions for PCR were carried out prior to amplification. The total reaction volume was 25 µl containing 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 10 pM of primer, 2.5 mM dNTP mix and 150 ng genomic DNA and 0.5 units of Taq DNA *polymerase* (Bangalore Genei Pvt. Ltd).

RAPD primers

The primers (Series-A and H) were selected after screening 40 random decamer primers (Integrated DNA Technology of Biogene, USA). Amplifications were performed in a thermocycler (Eppendorf Mastercycler Gradient) using the cyclic parameters: initial denaturation at 95 ^{o}C for 5 min followed by 35 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 37 ^{o}C for 2 min, extension at 72 ^{o}C for 2 min and final extension at 72 ^{o}C for 10 min and final hold at 4 ^{o}C.

Dwarf specific primers

The regenerated plants were also analyzed using a dwarf specific SCAR marker with the sole objective of detecting them at an early stage of development as described in the work of Damasco et al. (1996). In a multiplex PCR, the dwarf (Dw) specific primers (Dw1-5´CTGTG-GTTGCATTCTCATAC3´ and (Dw2 - 5´CTGAATCATACT GCGAACC3´) and an extra pair of primers to amplify 18S rRNA gene (18SF 5´CATCACAGGATTTCGGTCCT3´ and 18SR 5´AGACAAATCGCTC-CACCAAC3´) of *Musa acuminata* (Gene bank accession number U42083.1) were used (Ramage et al., 2004). DNA amplifications were performed in a total of 20 µl with 1X PCR buffer (10 mM Tris-HCl, 4.5 mM MgCl2, 50 mM KCl, pH 8.3), 0.25 mM dNTP's, 500 nM B1/B2 SCAR primer, five different concentrations of *Musa* 18S primers (25, 50, 100, 125 or 250 nM, dependent on run), and 3 units of Taq DNA polymerase. The amplification reactions were heated to 94 °C for 5 min followed by 30 cycles each of 95 °C for 30 sec, 56 °C for 1 min, 72 °C for 2 min with a final extension at 72 °C for 3 min. and a final hold at 4 °C.

Gel electrophoresis and scoring of polymorphism

The amplified products were subjected to agarose gel electrophoresis

18

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

by running the amplified products on 1.5% agarose gel in 1X TAE buffer. The presence of polymorphism was scored visually. The data were used to calculate Jaccard's (1908) similarity coefficients. The similarity measures were subjected to unweighted pair group method analysis using arithmetic averages (UPGMA).

## RESULTS AND DISCUSSION

Induction of somatic embryogenesis

Development of embryogenic cell suspensions (ECS) is an important target for large-scale propagation and genetic manipulation in banana. Somatic embryogenesis from immature male inflorescences (IMFs) is the method of choice. In order to extend the technique to other commercial cultivars, however, studies need to be focused on different genomic groups and optimize their response to *in vitro* culture and plant regeneration. In the present study, the commercially important banana cultivar 'Lal Kela' (genomic group AAA) was used to establish embryogenic cultures using immature male inflorescences explants. The IMF explants become brown at the base within a week of culture. These explants showed swelling in the next few weeks followed by the development of globules on medium supplemented with 2,4-D (Fig. 1a). Upon transfer to fresh M1 medium, white tissue protruded from the inflorescence tissues within 2 months after culture initiation (Fig. 1b, c). The white tissues were excised from the mother tissue and transferred to M2 medium where compact white callus, non-friable embryogenic tissues and early stages of embryos were formed within 2 weeks of the transfer. The cultures also displayed different morphogenic responses such as: the formation of yellow nodular callus, explant engulfed with callus development, nodular to creamish white callus, and appearance of somatic embryos (Fig. 1c). In terms of callus induction 'Lal Kela' showed the highest frequency induction (77.7%) on M1 medium. Embryogenic callus induction was 83.3%. This is in contrast to other banana cultivars belonging to different genomic groups which showed lower frequency of embryogenic callus induction (Meenakshi et al., 2007).

Induction and growth of cell suspension cultures

Upon transfer to liquid culture medium, the friable embryogenic callus released embryogenic cells with dense cytoplasm. Suspension cultures consisted of a heterogeneous mixture of different kinds of cells and cell clusters. Initially, these cultures were very heterogeneous and contained large translucent cells as well as small dense cells. Upon frequent subculture at 3-4 day intervals, and subsequently at 2-week intervals, the suspension cultures became more uniform and consisted only of clusters of small tightly packed cells with a dense cytoplasm. Secondary somatic embryogenesis was also observed (Fig. 1d,e,f) characterized by typical globular stage embryos directly from

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

19

primary somatic embryos which oc- curred in clusters, initially appearing
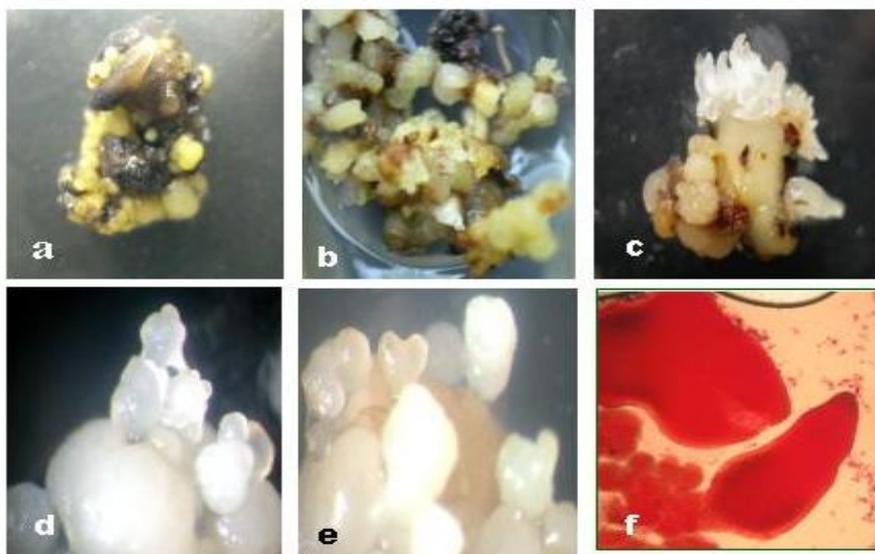
Figure 1. Induction of somatic embryogenesis in banana 'Lal Kela'
a – initiation of yellow nodular callus on M1 medium, b – nodular to creamish white callus on M2 medium, c – embryo development, and d, e, f – microscopic view of somatic embryos after squash preparation

as small, hyaline protuberances on the surface of the clusters. The embryos developed with a green plumule (Fig. 2).

The growth of the cell cultures was monitored daily (Fig. 3) and it fit a linear regression equation. It was observed that the final culture weight increased by 0.0384 units with each day of culturing (Fig. 4). The coefficient of determination R^2 showed that 92.84% variation in final weight was due time of culturing. (The growth rate over the initial growth rate was calculated even for the interval days. The percent growth rate over the initial growth rate was 125.88%, the percent growth rate over the average growth rate was 166.95% and the compound growth rate was 0.01945, which was the increase in growth per day. Statistically as per the linear regression curve, equation of intercept, and slope, the BO = 15.21, F = 25.70, SE = ±50.10 was calculated and was statistically significant.

In the present study, the growth of ECS in terms of cell proliferation (FW) and number of embryogenic clumps, indicated an increase in growth over a period of 2 days and a continuous proliferation rate. For example, the 4th day displayed an increase of 4.8 times, the 6th day had an increase of 9.7 times and the 7th day almost exhibited 10 times over the control (0th day). The results

20

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

Figure 2. Development of embryos (above) and stages in the plant development (middle) and regenerated plants (bottom)
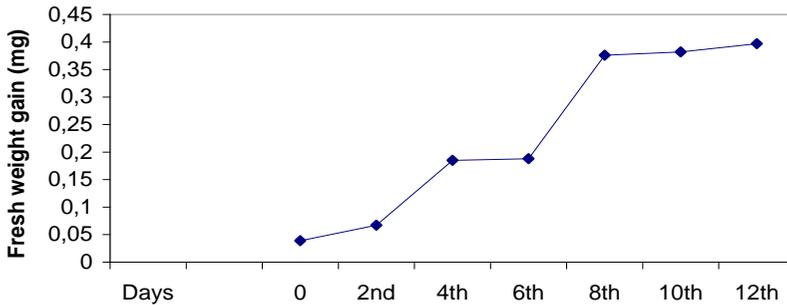
Figure 3. Time course of the growth of embryogenic cell suspension of banana 'Lal Kela'
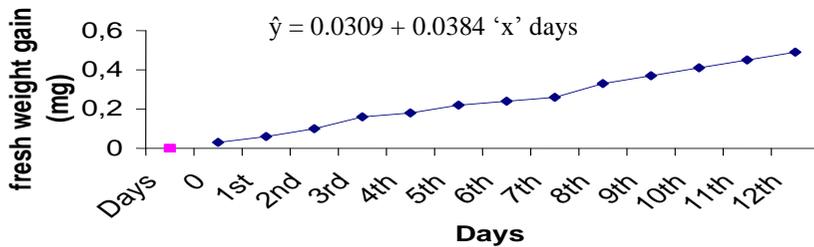
Figure 4. Linear regression curve showing unit growth per day of embryogenic cell suspension of banana 'Lal Kela'

indicated continuous proliferation of ECS and development of embryogenic cells/ clumps. During the culture period of 0 to 7 days, the number of embryogenic clumps in each size category also increased, suggesting the development of highly proliferative ECS. Georget et al. (2000) conducted a morphohistological study of the different constituents of embryogenic cell suspension in cv. Grande Naine, and classified ECS constituents into five types. Kosky et al. (2002) developed ECS for large scale growth in temporary immersion systems and showed that different cell densities gave the best results of somatic embryo development with an initial inoculum of 100 mg and embryo diameter from 0.5 to 1.2 mm. Xu et al. (2005) found that an established cell suspension grown over 1 and 2 weeks, yielded a 144.4% and 222.2% increase. Growth during the second week relative to growth during the first week was 153.9% indicating that the stationary growth phase had not yet been reached.

22

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

Plant regeneration

For the development of complete plantlets, globular stage embryos were transferred to different levels of BAP medium. The best response in terms of percentage of embryos showing shoot (50%) and root differentiation (44%) was observed on 0.5 µM BAP. However, a lower (0.02 µM) concentration showed reduced conversion frequency as the embryos developed only roots and light-brown, fragile embryos ceased to grow further. On the other hand, higher concentrations (10 µM to 20 µM) showed complete conversion with shoot and root development. Somatic embryos were vitrified on culture with high BAP concentration (beyond 20 µM) and the embryos become malformed and dried after 35 days (Tab. 1). The well-developed somatic embryo-derived plantlets ("emblings") grew normally during hardening in the green house (Fig. 2 c,d,e).

The features of somatic embryos (translucent and whitish) obtained in this study were similar to those obtained earlier by Côte et al., 1996; Escalant et al., 1994 and Strosse et al., 2006. In general, higher numbers of somatic embryos per flower cluster were obtained in 'Lal Kela' (4-10). The embryos passed through different developmental stages and finally green plumules emerged from them. Somatic embryos developed into the plantlets on ½ strength MS basal medium with 100 mg l^{-1} ME + 0.1% AC and 0.2% gelrite. The time span for the development of shoot and root was within 6 to 8 weeks. After this, the plantlets were transferred to a greenhouse for further growth. Khalil et al. (2002) obtained approximately 90% germination for development of somatic embryos into plantlets, and these were subcultured onto MS medium plus 0.1% activated charcoal 1 mgl^{-1} BA and 1 mgl^{-1} IAA where complete plantlets developed. Morphologically normal banana plants developed from all the regenerated plantlets, the first of which were produced within 6 months of culture initiation. In *Musa acuminata* 'Mas' (of AA genomic group), plant regeneration from embryogenic suspension cultures was achieved (Jalil et al., 2003). Embryogenic callus was obtained at a percentage of 10%. Plantlets were obtained on medium supplemented with 0.8 µM BA, at an average regeneration rate of 13. Xu et al. (2005) studied the establishment of cell suspension cultures of 'Williams', in which homogenous suspensions with more than 80% embryogenic clusters showed high regeneration capacity.

T a b l e 1. Effect of BAP on the development of somatic embryos to plantlets in the 'Lal Kela'

Treatment BAP [mg/l]	No. of embryos showing root and shoot	No. of embryos showing only shoots	No. of embryos showing only roots	Shoot length [cm]	Root length [cm]
MS+0.02	15	0	5	0.62±0.003	0.37±0.003
MS+0.5	38	0	0	1.76±0.002	0.86±0.05
MS+10	23	0	0	1.18±0.004	0.45±0.003
MS+15	26	0	0	0.87±0.003	0.58±0.05
MS+20	0	23	0	0.62±0.006	0

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

23

Molecular analysis of regenerated plants of 'Lal Kela' using RAPD and SCAR markers

During *in vitro* culture, culture induced variation usually results in off-types that reduce the commercial value of elite clones. Characterization of such variation is an important pre-requisite in the tissue culture of economically important plants. Martins et al. (2004) and Ramage et al. (2004) suggested the use of molecular markers in evaluating culture induced variation. In banana, occurrence of somaclonal variation resulting in dwarf off types has been reported (Israeli et al., 1995). Such dwarf somaclones were relatively stable and did not generally revert to a normal phenotype.
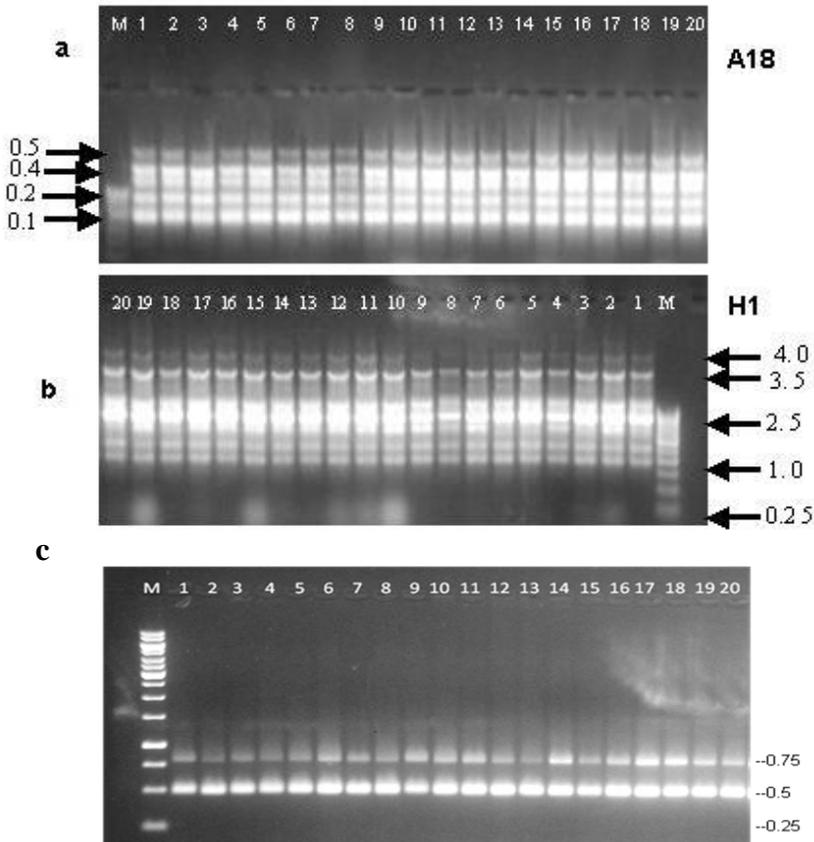
RAPD markers

The variation among the somatic embryo derived regenerants (LK1 to LK20) was analyzed using 20 selected random decamer primers (A and H series) (Fig. 5a,b). Of the 20 selected primers tested, a total of 1614 bands were obtained, out of which 240 bands were polymorphic. On average, each primer produced 3-7 bands with amplicon size ranging from 0.1 kb to 2 kb. The primers, in general, exhibited monomorphic bands and some minor polymorphic bands (in the case of primer A5, A15, H5, H11 and H12). Coefficient of similarity of 1.00 in respect to LK 20 and all the regenerants were close in sharing a similarity index ranging from 0.97 to 0.81 (Fig. 6). LK12, LK18 and LK19 with a similarity index of 0.97 were close to LK 20.

Dwarf specific primers

The 21 regenerants were also analyzed using dwarf-specific SCAR marker to detect the dwarf off types, if any, at the *in vitro* level. The SCAR primers along with the 18S primer (as an internal control) were used in a multiplex PCR. The genomic DNA of the 21 regenerants analyzed with the dwarf primer, showed a specific band of 700 bp along with a 500 bp band as PCR control corresponding to 18S primer (Fig. 5c).

In the present study, both PCR based techniques (RAPD and SCAR) were used for assessing genetic variation among the regenerated plants. No major variation was observed among the regenerants as can be seen by monomorphic bands, by using the A and H series of RAPD primers. The results are in line with the study of Ray et al. (2006), that in *Musa* the extent of instability caused by *in vitro* culture of shoot tips was related to cultivar rather than culture condition. Venkatachalam et al. (2007) used two types of PCR based markers: RAPD and ISSR for the identification of somaclonal variants. The use of two types of markers, which amplify different regions of the genome, allows for better chances of identifying genetic variation in the plantlets. Although this study has not detected any genetic change, it is possible that some changes might have occurred that go undetected, as there is a possibility of point mutations occurring outside of the priming sites. This result can be expected since the 'Lal Kela' is triploid and the variation may occur in

24

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

Figure 5. Profiling of regenerants of banana 'Lal Kela' using RAPD markers (a, b) and SCAR marker (c). M-molecular weight and values are in kb; 1-parent, 2-20 regenerated plants

less than three copies of the chromosomes. Such findings are similar to those reported earlier by Walther et al. (1997).

In banana, dwarf variants are a common occurrence and an early identification of dwarf off types is essential before plants are made ready for field establishment (Smith and Hamill, 1993). Although morphological features provide reliable measurements for discrimination of off-types, the differences can be seen

at week 7 when the plants are 18-20 cm tall, ready for field establishment. In this regard, molecular markers can prove to be useful for the detection of the dwarfs at an early stage in tissue culture (Damasco et al., 1996, 1997; Ramage et al., 2004). SCARs are advantageous over RAPD markers as they detect only a single locus, their

Figure 6. Similarity coefficients among 20 regenerants of 'Lal Kela' based on RAPD primers (A and H) using Jaccard's coefficient of correlation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1																			
2	0.87	1																		
3	0.88	96	1																	
4	0.81	0.91	0.9	1																
5	0.92	0.94	0.92	0.88	1															
6	0.95	0.89	0.9	0.83	0.95	1														
7	0.83	0.87	0.9	0.81	0.83	0.88	1													
8	0.81	0.84	0.88	0.88	81	0.85	0.92	1												
9	0.89	0.92	0.91	0.84	0.87	0.91	0.91	0.87	1											
10	0.89	0.95	0.94	0.87	0.89	0.91	0.91	0.87	0.97	1										
11	0.89	0.95	0.94	0.87	0.89	0.87	0.87	0.82	0.95	0.95	1									
12	0.88	0.96	0.95	0.88	0.9	0.88	0.88	0.83	0.94	0.96	0.98	1								
13	0.89	0.9	0.91	0.82	0.84	0.87	0.91	0.89	0.9	0.92	0.9	0.91	1							
14	0.91	0.95	0.91	0.87	0.89	0.89	0.87	0.84	0.95	0.95	0.95	0.94	0.92	1						
15	0.92	0.94	0.92	0.85	0.88	0.9	0.88	0.85	0.96	0.96	0.96	0.95	0.94	0.98	1					
16	0.88	0.89	0.9	0.81	0.83	0.85	0.9	0.88	0.89	0.91	0.89	0.9	0.98	0.91	0.92	1				
17	0.88	0.96	0.95	0.88	0.9	0.88	0.88	0.83	0.94	0.96	0.98	1	0.91	0.94	0.95	0.9	1			
18	0.85	0.96	0.95	0.88	0.9	0.85	0.88	0.83	0.91	0.94	0.94	0.95	0.91	0.91	0.9	0.9	0.95	1		
19	0.88	0.96	0.92	0.88	0.9	0.85	0.85	0.81	0.91	0.94	0.96	0.97	0.91	0.94	0.92	0.9	0.97	0.97	1	
20	0.88	0.96	0.92	0.88	0.9	0.85	0.85	0.81	0.91	0.94	0.96	0.97	0.91	0.91	0.94	0.92	0.9	0.97	0.97	1

26

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

amplification is less sensitive to reaction conditions, and they can potentially be converted into codominant markers (Paran and Michelmore, 1993). The *in vitro* plants analyzed using the SCAR marker produced a characteristic pattern with an expected amplified fragment (1500 bp) indicating their dwarf off type (Damasco et al., 1996). In the present study, the specific band was not detected, but a 700 bp band was observed in all the regenerants, which possibly indicates some kind of deletion in the genomic region during the evolution of the Indian cultivars. That the absence of amplification was not due to failure of PCR amplification conditions was authenticated by an internal control using 18S rRNA primer. Reports on the profiling of dwarf and normal types with differential AFLP, TEAFLP and MSAP patterns also indicated different levels of polymorphism (around 25%) depending on the method, the primer combination, and the genotype (Engelborghs et al., 2007).

Taken together, the results report successful induction of embryogenic cultures and plant regeneration in the Indian banana 'Lal Kela' and assessment of variation using molecular markers among the regenerated plants. The somatic embryogenesis method may become useful for its exploitation in mutagenesis or genetic transformation aimed at the improvement of banana.

REFERENCES

Becker D.K., Dugdale B., Smith M.K., Harding R.M., Dale J.L. 2000. Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. Grande Naine via micro-projectile bombardment. PHYSIOL. PLANT. 97: 285-290.

Côte F.X., Domergne R., Monmarson., Schwendiman J., Teisson C., Escalant J.V. 1996. Embryogenic cell suspensions from male flower of *Musa* AAA cv. Grand Naine. PLANT CELL REP. 19: 229-234.

Damasco O.P., Graham G.C., Henry R.J., Adkins S.W., Smith M.K., Godwin I.D. 1996. Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas. PLANT CELL REP. 16(1-2): 118-123.

Damasco O.P., Adkins S.W., Godwin I.D., Smith M.K. 1997. Use of a SCAR based marker for the early detection of dwarf off- types in micropropagated Cavendish bananas. ACTA HORT. 461: 157-164.

Engelborghs I., Sagi L., Swennen R. 2007. Early detection of dwarf off types in banana (*Musa* sp.) using AFLP, TE-AFLP and MSAP analysis. In: Mohan Jain S., Swennen R. (eds.), Banana improvement: Cellular, molecular biology and induced mutations. Science Publ., USA, pp. 331-340.

Escalant J.V., Teisson C., Cote F.X. 1994. Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivar (*Musa* spp.). IN VITRO CELL. DEV. BIOL. 30: 181-186.

Ganapathi T.R., Suprasanna P., Bapat V.A, Kulkarni V.M, Rao P.S. 1999. Somatic embryogenesis and plant regeneration from male flower buds in banana. CURR. SCI. 79: 1229-1231.

Ganapathi T.R., Chakrabarti A., Suprasanna P., Bapat V.A. 2002. Genetic transformation in banana. In:

Jaiwal P.K., Singh RP (eds.), Plant Genetic Engineering, Vol. 4. Improvement of vegetables and fruits. Sci-Tech. Publishers, Texas USA, pp. 83-105.

Georget F., Domergue R., Ferrier N., Cote F.X. 2000. Morphohistological study of the different constituents of a banana (*Musa* AAA, cv. Grand Naine) embryogenic cell suspension. PLANT CELL. REP. 19: 748-754.

Israeli Y., Reuveni O., Lahav E. 1995. *In vitro* Culture of Bananas. In: Gowen S. (ed.), Bananas and Plantains, Chapman and Hall, London, UK, pp. 147-17.

Jalil M., Khalid N., Othman R.Y. 2003. Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). PLANT CELL. TIS. ORG. CULT., 75: 209-214.

Khalil S.M., Cheah K.T., Perez E.A., Gaskill D.A., Hu J.S. 2002. Regeneration of banana (*Musa* spp. AAB cv. 'Dwarf Brazilian') via secondary somatic embryogenesis. PLANT CELL REP. 20: (12): 1128-1134.

Kosky R.G., Silva M.D., Perez L.P., Gilliard T., Martinez F.B., vega M.R., Milian M.C., Mendoza E.Q. 2002. Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled up in a bioreactor. PLANT CELL TISS. ORG. CULT. 68: 21-26.

Kulkarni V.M., Suprasanna P., Bapat V.A. 2006. Plant regeneration through multiple shoot formation and somatic embryogenesis in a commercially important and endangered Indian banana cv. Rajeli. CURR. SCI. 90(6): 842-846.

Ma S.S. 1991. Somatic embryogenesis and plant regeneration from cell suspension culture of banana. Proceedings of symposium on tissue culture of horticultural crops. Taibei, Taiwan, 8-9 March, 1988, pp. 181-188.

Martins M., Sarmento D., Oliveira M.M. 2004. Genetic stability of micropropagated almond plantlets as assessed by RAPD and ISSR markers. PLANT CELL. REP. 23(7): 492-496.

Meenakshi S., Shinde B.N., Suprasanna P. 2007. Somatic embryogenesis, plant regeneration and genetic fidelity testing of regenerants in different banana cultivars. National conference on production and utilization of banana for economic livelihood and nutritional security. October 25-28, 2007. National Research Centre on Banana, Trichy, India. (Abst.), pp. 52.

Menon R. 2000. Preliminary evaluation of some banana introductions in Kerala (India). Inf. Musa 9(2): 27-28.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. PHYSIOL. PLANT. 15(3): 473-497.

Navarro C., Escobedo R.M., Mayo A. 1997. *In vitro* plant regeneration from embryogenic cultures of a diploid and a triploid, Cavendish banana. PLANT CELL TISSUE ORGAN CULT. 51: 17-25.

Panse U.G., Sukhatme P.V. 1985. Statistical methods for agriculture workers, published by I.C.A.R. New Delhi.

Paran I., Michelmore R.W. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. THEOR. APPL. GENET. 85: 985-993.

Ramage C.M., Borda A.M., Hamill S.D., Smith M.K. 2004. A simplified PCR test for early detection of dwarf off-types in micropropagated Cavendish bananas. SCI. HORT. 103(1): 145-151.

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

28

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

PLANT CELL TISSUE ORGAN CULT 85(1): 11-21.

Rohlf F.J. 1990. NTSYS. Pc Numerical taxanomy and multivariate analysis system, version 2.02. App. Biostat., New York.

Schenk R.U., Hildebrandt A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. CAN. J. BOT. 50: 199-204.

Singh H.P. 2007. Research and development of banana and plantain in India. National conference on banana, pp. 1-19.

Smith M., Hamill S. 1993. Early detection of dwarf off-types from micropropagated Cavendish bananas. AUST. J. EXP. AGRIC. 33: 639-644.

Smith M.K., Hamill S.D., Becker D.K., Dale J.L. 2005. *Musa* spp. banana and plantain. In: Litz R.E. (ed.), Biotechnology of Fruit and Nut crops. CAB International Wallingford, UK, pp. 366-391.

Stewart C.N. Jr., Via, L.E. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14: 748-751.

Stover R.H. 1980. Sigatoka leaf spots of bananas and plantains. PLANT DIS. 64: 750-755.

Strosse H., Schoofs H., Panis B., Andre E., Reyniers K., Swennen R. 2006. Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). Plant Sci. 170: 104-112.

Suprasanna P., Panis B., Sági L., Swennen R. 2002. Establishment of embryogenic cell suspension cultures from Indian banana cultivars. 3rd and Final Research Coordination Meeting of the FAO/IAEA on Cellular biology of banana. Belgium, pp. 52-53.

Venkatachalam L., Sreedhar R.V., Bhagyalakshmi N. 2007. Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. Plant Growth Regul. 51: (3): 193-205.

Walther A., Ilan A., Lerer A.D., Khayat E. 1997. Analysis of somaclonal variation in tissue cultured banana plants (*Musa* AAA cv. 'Grand Nain'). ACTA HORT. 447: 379-383.

Xu C.X., Panis B., Strosse H., Li H.P., Xiao H.G., Fan H.Z., Swennen R. 2005. Establishment of embryogenic cell suspensions and plant regeneration of the dessert banana 'Williams' (*Musa* AAA group). J. HORT. SCI. BIOTECH. 80: 523-528.

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30

29

EMBRIOGENEZA SOMATYCZNA Z NIEDOJRZAŁYCH KWIATÓW MĘSKICH I ANALIZA MOLEKULARNA ZREGENEROWANYCH ROŚLIN BANANA ODMIANY 'LAL KELA' (AAA)

Sidha Meenakshi, Bansi Narayanrao Shinde
i Penna Suprasanna

S T R E S Z C Z E N I E

Rozwój kultur embriogenicznych mających wysoką zdolność regeneracyjną i pochodzących z odmian bananów ważnych pod względem handlowym, jest niezbędny do manipulacji genetycznej i rozmnażania *in vitro*. Badania miały na celu indukcję somatycznej embriogenezy z męskich niedojrzałych kwiatostanów banana odmiany Lal Kela (czerwony banan) wyszczepionych na pożywce M2 (Ma i in., 1991) wzbogaconej 2,4-D. Zawiesina kultur komórkowych powstała z wytworzonego kalusa wytworzyła wczesne stadia embryonalne. Zarodki somatyczne rozwinęły się w roślinki na pożywce podstawowej MS dwukrotnie i uzupełnionej 100 mg l^{-1} ME + 0.1% AC i 0.2% gelritu. Rośliny zregenerowane w wyniku embriogenezy somatycznej wykazały niewielkie zróżnicowanie, które określone było z użyciem markerów RAPD i SCAR

Słowa kluczowe: embriogeneza somatyczna, banan, *Musa*, zgodność genetyczna, regeneracja roślin

30

J. Fruit Ornam. Plant Res. vol. 19(2) 2011: 15-30