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ARTICLE

RAPD analysis of somaclonal variation in banana (*Musa acuminate* L.) cultivar Valery

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ABSTRACT Thirty decamer RAPD primers were used to study somaclonal variation among the parental plants as well as regenerated plants of the first, third, fifth, seventh and ninth sub-cultures. Eighteen out of thirty primers produced 289 bands in all the genotypes studied. Hundred and forty-two bands (48.95%) were common in the parental genotype and the re-generated plants while 147 bands were polymorph (51.40%). Among the primers used, OPI-07 produced the highest number of bands (24) while primers OPH-16 produced the lowest number (5). In total 74 specific bands were observed in the parental genotype and the regenerated plants of the sub-cultures. The presence of specific band/loci in the parental plants and loss of it in the regenerated plants of different sub-cultures indicates the loss of certain loci during tissue culture due to somaclonal variation. Such specific loci are of high importance in the genetic identification of the genotypes or somaclones from each other. Grouping of the parental cultivar and their sub-cultures regenerated plants indicate the genetic distinctness of the genotypes studied as they are placed in different clusters/groups far from each other. It also seems that the genetic variations induced in the regenerated plants increase with the time-period of the sub-culture.
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KEY WORDS

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Banana (*Musa acuminate* L.) with its different cultivars is cultivated in many tropical and semi-tropical countries and is considered a major staple food as well as an export commodity. Bananas are propagated vegetatively through suckers. Since most of the edible bananas are triploid and are nearly sterile and parthenocarpic, using conventional breeding methods for their improvement are difficult and cumbersome. Different approaches including mutation breeding and bio-technological methods have been applied to improve banana cultivars (Banerjee and De Langhe 1985; Wong 1986; Novak et al. 1989; Suprasanna et al. 2001, 2008).

One of the sources for inducing genetic variability in crop plants including banana is somaclonal variation. Plant tissue culture leading to somaclonal variation has been considered as a rapid and reliable approach for improvement of plants as the generated variation can be used either directly or in-directly in a breeding program aimed at crop improvement (Jain 2000) and has been used in banana cultivars to obtain superior quality banana clones (Maria and Garcia 2000; Asif and Mak 2004; Hwang and Ko 2004).

Somaclonal variation is used to describe the occurrence of genetic variants derived from *in vitro* procedures; it is also called tissue or culture-induced variation (Soniya et al. 2001). Such variation arises in tissue culture as a manifestation of epigenetic influence or a change in the genome of differentiating vegetative cells induced by tissue culture and is expected to generate stable plants carrying interesting heritable traits (Soniya et al. 2001). Four critical variables for somaclonal variation: genotype, explant origin, cultivation period and the cultural condition in which the culture is made.

The molecular markers are extensively used in germplasm characterization, fingerprinting, genetic analysis, linkage mapping, and molecular breeding. These markers are also used in identification of possible somaclonal variants at an early stage of development which is considered very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variants (Soniya et al. 2001). RAPD (Random Amplified Polymorphic DNA) analysis using PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals. The advantages of this technique are: a) a large number of samples can be quickly and economically analyzed using only micro-quantities of material; b) the DNA amplicons are independent from the ontogenetic expression; and c) many genomic regions can be sampled with a potentially unlimited number of markers (Soniya et al. 2001).

Among molecular markers, RAPD markers are used widely in studying the genetic diversity of somaclonal variations in various plant species (Damasco et al. 1996, 1998; Soniya et al. 2001) including banana (Hernandez et al. 2007). Damasco

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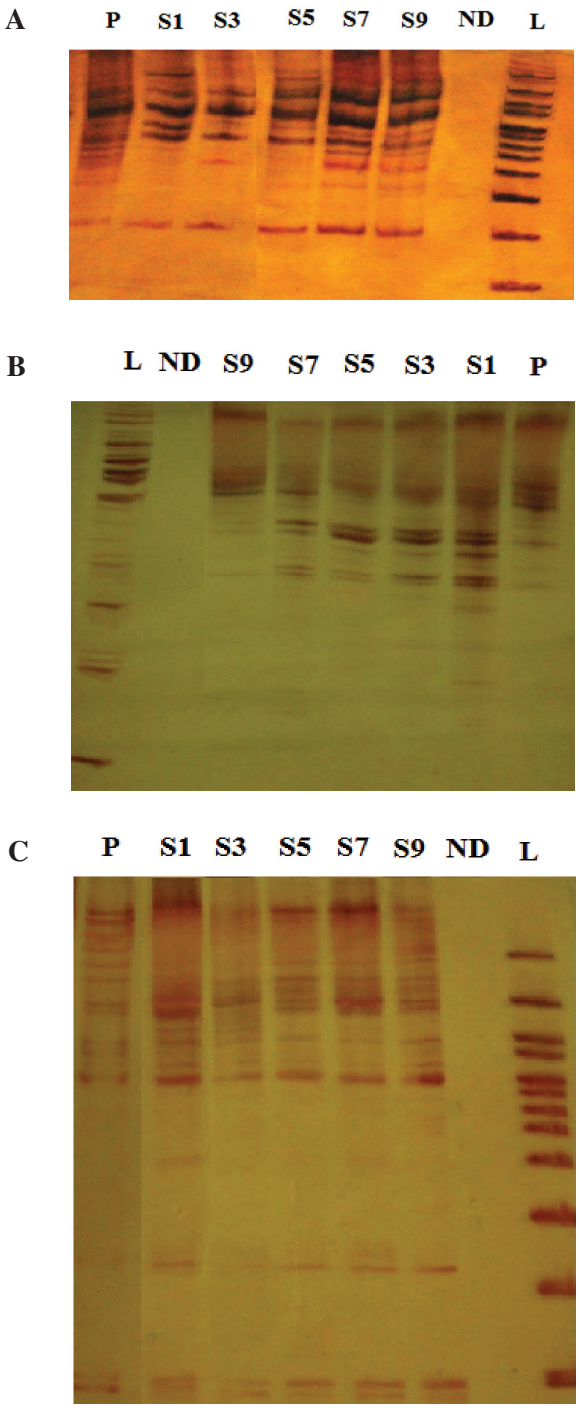

Figure 1. RAPD profile of primers OPI-07 (A), OPR-01 (B). and OPH-16 (C). Abbreviations: P = parental plants, S1, S3, S5, s7 and s9 = regenerated plants of the first, third, fifth, seventh and ninth sub-cultures respectively, ND = No DNA and L = 100 bp molecular ladder.

et al. (1996) compared normal and dwarfs banana and found a single RAPD band (OPJ-04 marker) in the normal but not in dwarf types. This was subsequently characterized into a SCAR (sequence characterized amplified region) for use as a PCR based detection system for dwarf types (Damasco et al. 1998). The present study reports the use of RAPD markers in studying the somaclonal variants obtained from tissue culture of banana cultivar Valery for the first time.

Materials and Methods

Tissue culture

Meristem-tip cultures of banana (*Musa acuminate* L.) cultivar Valery were derived from shoot apexes. Explants (ca. 10 x 10 x 6 mm) obtained from decapitated shoot apexes of suckers were surface sterilized by 70% ethanol for 20 seconds, then incubated in a 5% solution of sodium hypochloride for 20 min., followed by three rinses in sterile distilled water. The effects of cytokinins [Benzylaminopurine (BAP), kinetin (KIN) and N- phenyl – N´- 1,2,3 – thiadiazol 5-yl urea (TDZ)] combined with auxin [Indoleacetic acid (IAA)] were evaluated on basal Murashige and Skoog (MS, 11) medium. The pH was adjusted to 5.7 with 1 M NaoH before agar and charcoal was added. The cultures were maintained at 25ºC with 16 h photoperiod at a photosynthetic photon flux density of 120 µmol /m ²/ s. Sub-culturing was carried out at 45-day intervals. All treatments were performed on three replications of 10 explants in experiments employing a completely randomized design. The data on shoot number, shoot length and fresh weight of shoot were analyzed by ANOVA followed by Duncan's test (15).

DNA extraction and PCR amplification

Fresh leaves of five randomly selected plants were bulked for DNA extraction. DNA was extracted from the leaves of the parental genotype and regenerated plants of the first, third, fifth, seventh and ninth sub-cultures by using acetyltrimethyl ammonium bromide (CTAB)-based procedure. For RAPD analysis, the PCR reaction mixture consisted of 1 ng template DNA, 1 x PCR buffer (10 mM Tris-HCJ pH 8.8, 250 mM KC12), 200 µM dNTPs, 0.80 µm 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 µl. DNA amplification was performed on a palm cycler GP-00 1 (Corbet, Australia). Template DNA was initially denatured at 94°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92°C, primer annealing for 1 min at 36°C and primer extension for 2 min at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion.

Electrophoresis

The PCR amplified products were separated by electrophoresis on a 1.5% agarose gels using 0.5 X TBE buffer (44.5 mM Tris/Borate, 0.5 mM EDTA, pH 8) or 12% polyacrylamide gels. The gels were stained with ethidium bromide and visu-

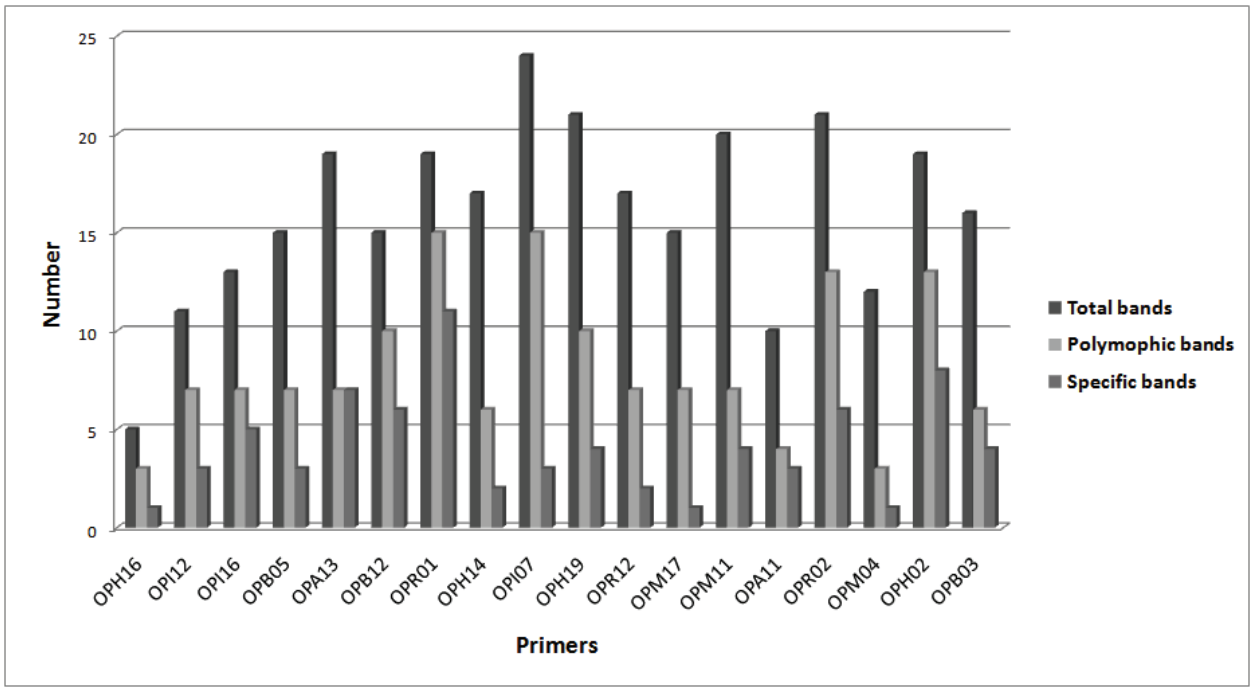

Figure 2. The number of total, polymorphic and specific bands produced by different primers in all parental and regenerated plants of different sub-cultures.

alized under UV light or silver stained for added sensitivity. RAPD markers were used named by primer origin, followed with the primer number and the size of amplified products in base pairs. Thirty random primers of Operon technology (Alameda, Canada) were used (Table 1).

The experiment was repeated for 3 times and reproducible RAPD bands were used for further analysis. The bands obtained were treated as binary characters and coded accordingly (presence =1, absence = 0). Jaccard similarity was determined among the genotypes studied to be used in clustering. The genotypes showing similarity in their RAPD characteristics were grouped by using UPGMA (Unweighted Paired Group with Arithmetic Average) and Neighbor Joining (NJ) methods as well as ordination based on principal coordinate (PCO) and principal components (PCA) analysis (Sheidai et al. 2008). NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) was used for statistical analyses.

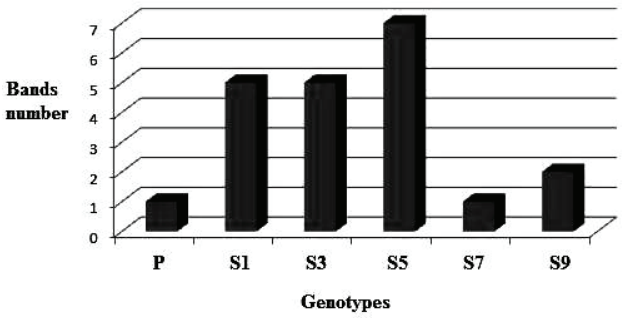

Figure 3. The number of specific bands produced by all primers in the genotypes studied.

Results

Tissue culture

TDZ promotes a higher number of shoots per explant compared to KIN, while, BAP shows intermediary results. However, the shoots developed in the presence of TDZ or KIN did not survive upon transferring. Moreover, in the absence of cytokinins, the entire shoot died within 2 weeks.

The presence of TDZ along with BAP and KIN significantly ($p< 0/05$) reduces the shoot elongation and shoots fresh weight which is in agreement with the results obtained in the order banana cultivars (Alvard et al. 1992). The number of shoots significantly increased with increasing concentration of TDZ in the media, but the elongation and fresh weight of shoots decreased significantly. The association of 0.15 mg/L TDZ and 2 mg/L IAA positively affected the multiplication of the banana cultivars, possibly due to its strong cytokinin activity (Nowak and Miczynski 2002).

At 2 mg/L concentration of BAP, the length of shoots and fresh weight of plantlets per explant was significantly increased compared to that of TDZ and KIN. The length of

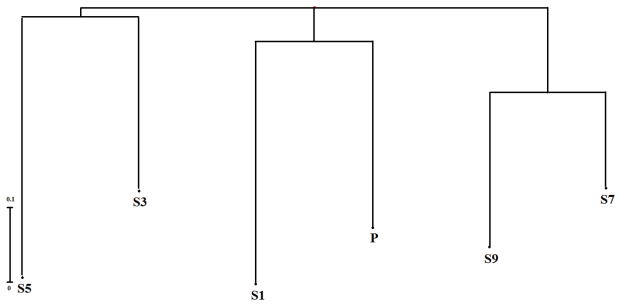

Figure 4. NJ clustering of the banana genotypes. Abbreviations: P = parental plants, S1, S, S5, S7 and S9 = regenerated plants of the first, third, fifth, seventh and ninth sub-cultures respectively.

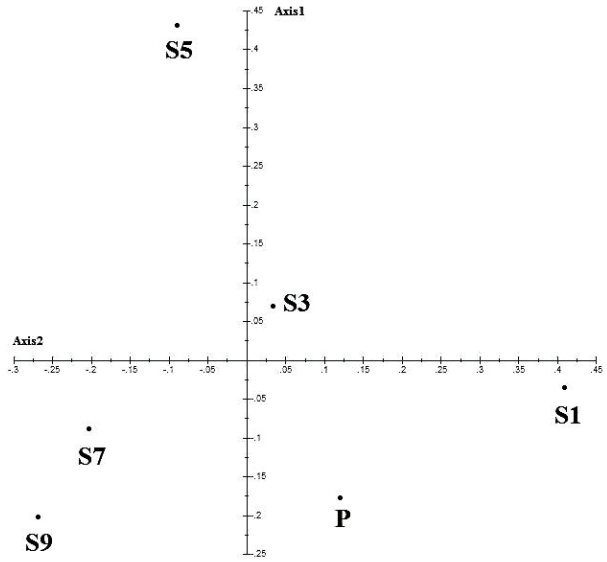

Figure 5. PCA ordination of the banana genotypes. Abbreviations: P = parental plants, S1, S, S5, S7 and S9 = regenerated plants of the first, third, fifth, seventh and ninth sub-cultures respectively.

shoots and fresh weight significantly increased with increasing concentration of BAP in the media. With 2 mg/L BAP and 1.5 mg/L KIN, a significant elongation of shoots as well as significant reduction of shoot proliferation and fresh weight occurred. At high concentration of BAP and KIN the number of shoots was significantly reduced. The final medium adopted included the salt formulation of Murashige and Skoog, 30 g/L of sucrose, N-phenyl-N–1, 2, 3- thiadiazol 5-yl Urea (0.5 mg/L) and Indoleacetic acid (2 mg/L). Under these conditions, a multiplication rate of 25 plantlets per explant was obtained in 120 days.

RAPD analysis

Eighteen RAPD primers out of 30, produced 289 bands (Figs. 1-3) in all the genotypes studied, out which, 142 bands (48.95%) were common in the parental genotypes and the regenerated plants of the sub-cultures while, 147 bands were polymorph (51.40%). Among the primers used, OPI-07 produced the highest number of bands (24) while primers OPH-16 produced the lowest number (5). The highest number of polymorphic bands (15) was observed in OPR-01 (84.20%) while the lowest number (3 bands) was observed in OPM-04 (25%).

Some of the RAPD bands/loci were present in all the genotypes except one, for example the band 1 of the primer OPH-16 was absent only in the regenerated plants of the fifth sub-culture, while bands 5, 8 and 9 of the primer OPI12 was absent only in the regenerated plants of the third sub-culture.

Some bands occurred only in one genotype and was absent in the others. The regenerated plants of the fifth sub-culture showed the highest number (7) of specific bands, while parental plants and the regenerated plants of the seventh sub-culture showed the lowest number (1) of the same. Band 1 of the primer OPI-16 occurred only in the regenerated plants of the first sub-culture, band 2 of this primer was specific for the regenerated plants of the fifth sub-culture while bands 4, 6 and 7 were specific for the regenerated plants of the first sub-culture. Similarly other primers also produced specific bands in different genotypes studied. Both clustering and ordination methods of the genotypes produced similar results separating the parental and regenerated plants in different clusters or groups (Figs. 4, 5).

Discussion

Variations observed in total number of RAPD bands as well as the number of specific bands among the parental plants and regenerated plants of different sub-cultures indicate genetic differences of the genotypes due to tissue culture and somaclonal variation induced. The presence of specific band/loci in the parental plants and loss of it in the regenerated plants of different sub-cultures indicates the loss of certain loci during tissue culture due to somaclonal variation, while the occurrence of specific bands/loci in the regenerated plants of different sub-cultures and their absence in mother plants may indicate the occurrence of genetic changes leading to formation of new binding sites in these plants. Such specific loci are of high importance in the genetic identification of the genotypes or somaclones from each other.

Since, even single base change at the primer annealing site is manifested as appearance or disappearance of RAPD bands, it could be suggested that tissue culture conditions have induced varied amount of genetic changes in different regenerated plants. Some of the molecular changes appeared identical in regenerated plants of different sub-cultures and appeared as non-parental bands. According to Soniya et al.

(2001), the reason for such commonness of genetic variation is that these plants are all derived from the same callus and the variations observed in the RAPD pattern may be due to different causes including loss/ gain of a primer annealing, due to point mutations or by the insertion or deletion of sequence or transposable elements (Peschke et al. 1991).

The occurrence of specific bands/loci in the regenerated plants of different sub-cultures shows genetic distinctness of these plants (somaclones) and the use of tissue culture in causing genetic variation in banana.

Grouping of the parental cultivar and their sub-cultures regenerated plants indicate the genetic distinctness of the genotypes studied as they are placed in different clusters/ groups far from each other (Figs. 4, 5). It also seems that the genetic variations induced in the regenerated plants increase with the time-period of the sub-culture. For example, the regenerated plants of the first sub-culture show comparatively lower degree of genetic difference from the parental plants as they are placed in the clusters much closer to each other compared to the regenerated plants of the latter sub-cultures. The findings here are in line with the earlier reports on application of RAPD in describing genetic polymorphisms among regenerated plants in several other plants including. *Apium* and *Prunus* species (Soniya et al. 2001) as well as in cotton (Sheidai et al. 2008).

Explant source is also considered as one of the critical variable for somaclonal variation. Since explants may present dissimilar regeneration rates, selection procedures can differ among different explants types. For example, plants regenerated from chrysanthemum petal epidermis-induced calli showed greater somaclonal variation than those from apex-induced calli (De Jong and Custers 1986). Therefore it may be suggested that different sources of explants may be tried in banana and compare the level of genetic variation obtained.

In conclusion, the results of present study show the occurrence of somaclonal variation due to different sub-culturing of banana cultivar Valery, and that with increase in time period of sub-culturing, possibly more genetic variation may occur. The study also shows the use of RAPD molecular markers in revealing somaclonal variation in banana and that the occurrence of specific bands/loci in the parental plants as well as in the regenerated plants of different sub-cultures may be used in the genetic identification of the genotypes or somaclones from each other.

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