



Genetic variation induced by tissue culture in Banana (*Musa acuminata* L.) cultivar Cavandish Dwarf

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Abstract

An efficient medium culture for clonal mass propagation was established for the propagation of banana (*Musa acuminata* L.) cultivar Cavendish Dwarf including 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). Fifteen decamer RAPD primers were used to study somaclonal variation among the regenerated plants of the first, third and fifth sub-cultures. Eight out of fifteen primers produced 143 bands in all the genotypes studied. Fifty-three bands (37.70%) were common in the parental genotype as well as in the regenerated plants while 90 bands were polymorph (63.30%). Twenty-five specific bands were observed in the parental genotype

and the regenerated plants of different sub-cultures which may be used in genetic discrimination of the genotypes. The presence of specific bands in regenerated plants indicates the rearrangement in certain loci during tissue culture due to somaclonal variation.

Key Words: Banana, somaclonal variation, RAPD.

Introduction

Banana is one of the most important fruits in the world, both as a staple food as well as a major export commodity for many tropical and sub-tropical countries. Bananas are propagated vegetatively through suckers. Since most of the edible bananas are triploid and are nearly sterile and parthenocarpic, the use of conventional breeding methods for their improvement are difficult and cumbersome. Mutation breeding and biotechnological methods can offer as useful tools for banana improvement. The extensive works on *in vitro* propagation of banana (Banerjee and De Langhe, 1985; Wong, 1986; Novak et al., 1989; Suprasanna et al., 2002; Suprasanna et al., 2008) provided opportunity for *in vitro* mutagenesis and selection in different banana cultivars.

Plant tissue culture leading to somaclonal variation has been considered as one of the possible sources of inducing genetic variability in crop plants to be used in breeding programs. Somaclonal variation is a rapid and reliable approach for improvement of plants as the generated variation can be used either directly or indirectly 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 et al., 2004; Hwang and Ko, 2004).

Somaclonal variation is used to describe the occurrence of genetic variants derived from *in vitro* procedures (Larkin and Scowcroft, 1981; Isabel et al., 1993), it is also called tissue or culture-induced variation (Kaeppeler et al., 2000; Bordallo et al., 2004). Such variation arises in tissue culture as a manifestation of epigenetic influence or changes 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). The critical variables for

somaclonal variation: genotype, explant origin, cultivation period and the cultural condition in which the culture is made (Soniya et al., 2001).

The molecular marker technologies have become a powerful tool in crop improvement through their use in germplasm characterization and fingerprinting, genetic analysis, linkage mapping, and molecular breeding. Identification of possible somaclonal variants at an early stage of development is considered to be 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 (Damasco et al., 1996; Maria and Garcia, 2000).

RAPD markers have been used widely in studying the genetic diversity of somaclonal variations in various plant species (Damasco et al., 1996; Maria and Garcia, 2000; Soniya et al., 2001) including banana (Hernandez et al., 2007). Damasco 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., 1996). The present study reports the use of RAPD markers in studying the somaclonal variants obtained from tissue culture of banana cultivar Cavendish Dwarf.

Material and Methods

Tissue culture

Meristem-tip cultures of banana (*Musa acuminata* L.) cultivar Cavendish Dwarf were derived from shoot apices. Explants (ca. 10 x 10 x 6 mm) obtained from decapitated shoot apices 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, 1962) medium. The pH was adjusted to 5.7 with 1 M NaOH before agar and charcoal was added. The cultures were maintained at 25 °C with 16 h photoperiod at a photosynthetic photon flux density of 120 $\mu\text{mol /m}^2\text{/ s}$. 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.

RAPD analysis

Fifteen decamer RAPD primers of Operon technology (Alameda, Canada) were used in molecular study. For RAPD analysis, fresh leaves were selected randomly from trees and DNA extraction was done by use of NucleoSpin Plant kit (Macherey-Nagel, Germany). The PCR reaction mixture consisted of 1 ng template DNA, 1 x PCR buffer (10 mM Tris-HCL pH 8.8,

250 mM KCL), 200 μ M dNTPs, 0.80 μ M 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 μ l. DNA amplification was performed on a palm cyclor GP-001 (Corbet, Australia). Template DNA was initially denatured at 92⁰C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92⁰C, primer annealing for 1 min at 36⁰C and primer extension for 2 min at 72⁰C. A final incubation for 10 min at 72⁰C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis on a 2% agarose gels using 0.5 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH 8.0) or 6% polyacrylamide gels. The gels were stained with ethidium bromide and visualized under UV light (Sambrook et al., 2001). A 100 bp DNA ladder (GeneRuler, Fermentas) was used as the molecular standard in order to confirm the appropriate RAPD markers. These markers were named by primer origin, followed with the primer number and the size of amplified products in base pairs.

The experiment was repeated for 3 times and reproducible RAPD bands were used for further analysis. Fragments, in the size range of 250 bp to 3000 bp were scored 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 ordination based on principal coordinate (PCO) as well as principal components (PCA) analysis (Sheidai et al., 2008). NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) was used for statistical analyses.

Results and Discussion

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 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 (1962), 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

Eight RAPD primers out of fifteen produced 143 bands (Figure 1) in all the genotypes studied. Out of 143 bands obtained, 53 (37.70%) were common in the parental genotypes and the regenerated plants of the sub-cultures while, 90 bands were polymorph (63.30%, Figure 2). Among the primers used, OPR-01 produced the highest number of bands (26) while primers OPH-19 produced the lowest number (9). The highest number of polymorphic bands was observed in OPA-13 (15 bands) while the lowest number was observed in OPH-19 (6 bands). In total 25 specific bands were observed in the parental genotype and the regenerated plants of the sub-cultures.

The primers OPH-14 and OPR-01 produced the highest number (6) of specific bands (Figure 3) while primers OPH-19 and OPA-15 produced the lowest number (4). The parental plants and the regenerated plants of the fifth sub-culture showed the highest number (5) of specific bands (Figure 4). The presence of specific bands/loci in the parental plants and loss of them in the regenerated plants of different sub-cultures indicates the loss of certain loci during tissue culture due to somaclonal variation.

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.

Thirty-four bands/loci were present in all the genotypes except one, for example the band 3 of the primer OPR-1 was absent only in the regenerated plants of the third sub-culture, while band 4 of the same primer was absent only in the regenerated plants of the first sub-culture.

Band 6 of this primer was absent only in the regenerated plants of the fifth sub-culture. Such cases were observed in the other primers too.

The absence of a band/ loci in one of the genotypes indicates genetic changes of the plants brought about during sub-culture. 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 these changes appeared identical in different plants as represented by appearance of non-parental bands. The reason for such commonness of genetic variation in these plants could be because they were all derived from the same callus (Soniya et al., 2001). 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).

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 (Figures 5 & 6). 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, viz. *Apium* species, and *Prunus* species (Soniya et al., 2001).

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. The present finding indicates the possible use of somaclonal variation as a source for inducing genetic variation in bana cultivars which may be used in planning breeding program of banana in the country.

References

Alvard D, Cote F, Tiesson C. (1992). Comparison of methods of liquid medium culture of banana micropropagation. *Plant Cell Tissue Org. Culture*. 32: 55-60.

Asif MJ, Mak C, Othman RY (2004). Study of resistance of *Musa acuminata* to *Fusarium oxysporum* using RAPD markers. *Biol.Plants*. 48: 93-99.

Banerjee N, De Langhe EA (1985). Tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). *Plant Cell Report*. 4: 351-354.

Damasco OP, Graham GC, Henry RJ, Adkins SW, Smith MK (1996). Random amplified polymorphic DNA (RAPD) detection of dwarf off types in micropropagated Cavendish bananas. *Acta Hort*. 461: 157-164.

De Jong J, Custers JBM (1986). Induced changes and flowering of *Chrysanthemum* after irradiation and *in vitro* culture of pedicels and petals epidermis. *Euphytica*. 35:137-148.

Hernandez R, Rodriguez R, Ramirez T, Canal MJ, Guillen D, Noceda C, Escalona M, Corujo M, Ventura J (2007). Genetic and morphoagronomic characterization of plantain variants of *Musa* AAB clone CEMSA. *J. Food. Agri. Environ*. 5: 220-223,

Hwang SC, Ko WH (2004). Cavendish banana cultivars resistant to *Fusarium* wilt acquired through somaclonal variation in Taiwan. *Plant Diseases*. 88: 580-588.

Jain SM (2000). Tissue culture induced variation in crop improvement. *Euphytica*. 118: 153-166.

Maria DCV, Garcia ED (2000). Analysis of a *Musa* spp. somaclonal variant resistant to yellow sigatoka. *Plant Mol. Biol. Report.* 18: 23-31.

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

Novak FJ, Afza R, Vanduren M, Perea-Dollas M, Conger BV, Xiaolang T (1989). Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) (*Musa* spp.). *Biotechnol.* 7: 154-159.

Nowak B, Miczynski K (2002). The course and efficiency of organogenesis on leaf explants of Plum Wegierka Zwyczajna (*Prunus domestica* L.) induced by cytokinins. *Elect. J. Polish Agri. Univ. Biotechnol.* 5: 120-124.

Peschke VM, Philip RL, Gengenbach BG (1991). Genetic and molecular analysis of tissue-culture derived Ac elements. *Theor. Appl. Genet.* 82: 121-129.

Sambrook J, Fritsch EJ, Maniatis T (2001) *Molecular cloning: A laboratory manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sheidai M, Yahyazadeh F, Farahanei F, Noormohammadi Z (2008). Genetic and morphological variations induced by tissue culture in tetraploid cotton (*Gossypium hirsutum* L.) *Acta Biol. Szeged.* 52: 33-38.

Soniya EV, Banerjee NS, Das MR (2001). Genetic analysis of somaclonal variation among callus-derived plants of tomato. *Curr. Sci.* 80: 1213-1215.

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. KUL, Leuven, Belgium, September 24-26, pp 9-10.

Suprasanna P, Sidha M, Ganapathi TR (2008). Characterization of radiation induced and tissue culture derived dwarf types in banana by using a SCAR marker. Aust. J. Crop Sci. 1: 47-52.

Wong WC (1986). In vitro propagation of banana (*Musa* spp.): Initiation, proliferation and development of shoot-tip cultures of defined media. Plant Cell Tissue. Organ Culture. 6: 159-166.

Table 1. The primers used and their nucleotide sequences.

Primer	Nucleotide sequence
OPA-11	5' CAATCGCCGT 3'
OPA-13	5' CAGCACCCAC 3'
OPA-15	5' TTCCGAACCC 3'
OPB-03	5' CATCCCCCTG 3'
OPB-05	5' TGCGCCCTTC 3'
OPH-07	5' CTGCATCGTG 3'
OPH-14	5' ACCAGGTTGG 3'
OPH-19	5' CTGACCAGCC 3'
OPI-07	5' CAGCGACAAG 3'
OPM-17	5' TCAGTCCGGG 3'
OPR-01	5' TGCGGGTCCT 3'
OPR-02	5' CACAGCTGCC 3'
OPR-06	5' GTCTACGGCA 3'
OPR15	5' GGACAACGAG 3'
OPM-19	5' CCTTCAGGCA 3'

Legends to Figures 1-6.

Fig. 1. RAPD profile of OPM-17.

Line 1-3 = Regenerated plants of sub-cultures 1, 3 and 5 respectively, line 4 = parental plants,

No = No DNA and M = Molecular ladder 100 bp.

Fig. 2. The primers producing polymorphic bands.

Fig. 3. The primers producing specific bands.

Fig. 4. The number of specific bands in the genotypes studied.

Abbreviations: P = parental plants, S1, S3 and S5 = regenerated plants of the first, third and fifth sub-cultures respectively.

Figs. 5 & 6. UPGMA clustering and PCA ordination of the banana genotypes.

Abbreviations: P = parental plants, S1, S3 and S5 = regenerated plants of the first, third and fifth sub-cultures respectively.





