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Morphological and Molecular Characterization of Some Banana Micro-propagated Variants

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ABSTRACT

Meristem tips were introduced and multiplied *in vitro* for sixth subculture. Regenerated plantlets were adapted in the greenhouse. Some variants were isolated at adaptation and nursery stages. Otherwise, the percentage of variation in adaptation stage was lower than in the nursery stage. Ten micro-propagated variants were isolated and described morphologically and analyzed by random amplified polymorphic DNA (RAPD) using three arbitrary 10-mer oligonucleotide sequences as compared to normal plant.

Key Words: Banana; Tissue culture; Shoot-tip culture; *In vitro* plantlets; RAPD; Somaclonal variation; ELISA

INTRODUCTION

Banana is one of the world's most important horticultural crops. Bananas (*Musa* spp.) are cultivated on five continents in about 120 countries. Current world production of banana is estimated at 97.5 million tonnes per year covering 10 million hactare (Kalloo, 2002; Singh, 2002). The production of this crop is limited by a wide range of diseases including fungal, viral, bacterial and nematode (Dale & Harding, 1998). Banana plantains are normally propagated vegetatively using suckers of various sizes or pieces of the corm since the fruits of the edible clones are seedless. During past, efforts have been made to improve propagation rates using conventional techniques (Berg & Bustomante, 1974; Vuylsteke & Delanghe, 1985). In the recent times, tissue culture methods play a significant role in plant improvement, either directly or as an adjunct to more traditional methods.

Plant tissue culture technique has great potential as a means of vegetative propagation of economically important species, especially for those difficult to propagate by conventional methods (seeds or cuttings). Banana is one of the main plants of horticultural interest, which are multiplied by micro-propagation, compared with the conventional planting methods. Micro-propagated banana plants have two major advantages: (1) their size and septic quality make easier to introduce new varieties into production areas and (2) their nematode-free status makes possible to improve soil parasite control and reduce the use of nematicides (Ganry, 1986; Navarro *et al*., 1994). In addition healthy plants can be grown in the laboratory at any time.

Tissue culture is important to establish and/or maintain a "virus-free" stock somatic hybridization induction and selection of mutants and germplasm conservation (Conger, 1980; Vuylsteke, 1989). *In vitro* culture techniques of banana plants can produce thousands of plants in a relatively shorter time either using somatic embryo (Cronauer & Krikorian, 1983) or apex explants (Cronauer & Krikorian, 1984b), which require different culture media for shoot multiplication and root differentiation (Cronauer & Krikorian, 1985, 1988). With the extensive use of *in vitro* techniques, somaclonal variation is commonly observed in *Musa* propagation (Vuylsteke *et al*., 1991) and the genetic origin of the phenotypic variation is a subject of more discussion. Shoot induction from sucker tips with N^6-benzyladenine (BA) is the most common method for *in vitro* propagation in *Musa* and the somaclonal variant index varies in a wide range between 0.1 and 60% depending on different parameters such as genetic mosaic, number of subcultures, growth regulator concentration, donor genotype etc. (Sandoval *et al*., 1996). Shepherd and Dos Santos (1996) have reported that *Musa* plants regenerated through *in vitro* culture with BA show a higher percentage of mitotic abnormalities than plants vegetatively propagated by classical methods. Sandoval *et al*. (1996) reported a somaclonal variant with mosaic-streaked leaves obtained from Grand Nain (AAA) cultivar. Trujillo and Garcia (1996) reported a somaclonal variant (CIEN BTA-03) resistant to Yellow Sigatoka (YS; *Microphaerella musciola Lench*) regenerated from adventitious shoots induced on excised shoot apices of Williams cultivar (susceptible to the disease), grown on MS medium (Murashige & Skoog, 1962) with 15 mg L^{-1} of N^6-benzyladenine (BA).

Somaclonal variation has also been evaluated at the DNA level in *Musa.* Kaemmer *et al*. (1992) used random amplified polymorphic DNA (RAPD) and micro-satellite fingerprinting to construct phylogenetic dendograms of *Musa* spp., and characterized a somaclonal variant of Grand Nain (AAA) named Novaria. Bhat *et al*. (1995) reported restriction fragment length polymorphism (RFLP) and RAPD studies in 57 *Musa* cultivars. Faure *et al*. (1993)

compared RFLP and RAPD markers as techniques for mapping analysis and concluded that RAPD markers are highly polymorphic for making maps. These complex patterns are ideal for comparing mutant genomes of the somaclonal variant CIEN BTA-03, or the Novaria somaclone (Kaemmer *et al*., 1992).

The objective of this work was to detect somaclonal variations in banana multiplied *in vitro* depending on the morphological characters and compare the DNA profiling of normal and variant plants with the aim to monitor the uniformity of plants multiplied *in vitro*.

MATERIALS AND METHODS

Plant material. Banana meristems of Williams cultivar were introduced from abroad in plastic containers and used as a stock for micro-propagation process to produce regenerated plantlets. The procedure for commercial micro-propagation of banana plantlets comprises four stages, culture initiation, multiplication (shooting), Rooting and acclimatization.

Commercial micro-propagation of banana plantlets. At initiation stage, the introduced meristems were sub-cultured on the initiated medium for 4 weeks. The cultures were incubated in the growth room under appropriate condition. At multiplication (shooting) stage, the individual multi-shoots (clusters) obtained from the previous stage were transferred to jars containing 40 mL of shooting medium. Cultures were incubated in the growth cabinet under appropriate conditions. This step was repeated six times at 4 weeks intervals. At rooting stage, shoots were cultured in jars containing 40 mL of rooting medium (with 7 - 8 shoots) and kept in the growth room under controlled conditions (Table I).

At adaptation stage, plantlets grown on rooting medium were removed from the culture jars and washed thoroughly with tap water to remove the aged root system. The plantlets were soaked in fungicide and transplanted into small plastic pots (6 cm diameter) containing a mixture of sand, peatmoss, vermiculite and covered with polyethylene tent to maintain high humidity. After two weeks, the cover was partially removed and plantlets kept in the greenhouse for eight weeks. These plantlets were transplanted to 20 cm plastic bags for 4 - 8 weeks. From the third week, all pots were fertilized with ¼-strength Hoagland solution twice every week and irrigation with water alone was applied every day.

Culture medium. Medium used was salt mix of Murashige and Skoog (1962) medium, 4.2 g L^{-1} constituent required in large quantities e.g., sucrose 30 g L^{-1} and agar 7 g L^{-1} were weighed at the time of medium preparation. Hormones requirement depended on each stage (Table I). All containers sealed with polypropylene caps and autoclaved under 1.5 b^{-2} at 121°C for 25 min. and then kept for three days at room temperature before culture.

Incubation condition. All cultures were incubated at 28 ± 1°C under photoperiod cycle of 16/8 h as light/dark. Light intensity was used at 25 klux, supplemented with white fluorescent tubes.

Isolation of the variants. Off-type of cv. Williams were isolated and kept either at adaptation stage or after transplanted in a big pots (25 cm width) (nursery stage) according to the morphological characters. Percentage of variations was recorded.

Virus detection. Presence of banana bunchy top virus (BBTV) and cucumber mosaic virus (CMV) was tested in the selected variants by direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) technique (Clark & Adams, 1977) using kits (Agadia Co., Indiana), as per manufacture's instructions.

Genomic DNA extraction. Genomic DNA (gDNA) was extracted on a mini-prep scale (Murray & Thompson, 1980). Small pieces (0.5 g) of leaf were frozen in liquid nitrogen in Eppendorf tubes and homogenized in 500 µL of extraction buffer (2% CTAB, 1.4 m NaCl, 20 mm EDTA pH 8.0, 100 mm Tris-HCl, pH 8.0, 0.1 m ß-mercaptoethanol). The extract was incubated at 60ºC for 20 min. To this 500 µL of phenol: chloroform: isoamyl alcohol (24: 24: 1) were added and mixed by vortexing for 30 sec followed by centrifugation at 10,000 x *g* for 5 min at room temperature. The aqueous phase was transferred to another tube and extracted again with 500 µL of chloroform: isoamyl alcohol (24: 1) in Eppendorf tube. To the aqueous phase, 0.6 volume of isopropanol were added, precipitated the gDNA, and spooled the fibrous gDNA. The gDNA was then washed three times with 70% ethanol, dried in vacuum, dissolved in TE containing 10 mg mL^{-1} RNase and incubated at 37ºC for 30 min., followed by extraction with phenol: chlorform: isoamyl alchol and the aqueous phase was transferred to a fresh tube. Thereafter, the gDNA was precipitaed by adding 0.3 m sodium acetate, pH 5.2 (final concentration) and 2.5 volume of ethanol and collected by centrifugation at 10,000 x *g* for 20 min at 4ºC. The pellet was washed with 70% ethanol, vacuum dried and dissolved in TE.

PCR condition. Three random oligonucleotide (10 mer) primers (Operon technologies Inc., Alameda, California) were designed for use in RAPD analysis. The primers were OPK01 (5′ TGC CGA GCT G 3′), OPK02 (5′ GTG AGG CGT C 3′), OPK03 (5′ CCC TAC CGA C3′). The PCR reactions were carried out in 50 µL volumes containing 100 ng of gDNA, 1.0 µm of each primer, 200 µm of dATP, dDTP, dCTP, dGTP, 10 mm tris-HCl, pH 8.3, 50 mm KCl, 1.5 mm $MgCl_2$ and 0.001% gelatine. The Taq DNA polymerase concentration was 1.5 units per assay. PCR reaction was conducted using a Perkin Elmer 2400 (Germany) thermocycler programmed for 45 cycles as follows: 94ºC/5 min (1 cycle), 94ºC/30 sec, 36ºC/30 sec, 72ºC/2 min (45 cycle) and 72ºC/7 min (1 cycle), then held at 4ºC. The PCR products were size-separated by gel electrophoresis (Pharmacia G N. 100 Submarine) on 1% agrose gels with 1 x TBE buffer and stained with ethidium

Table I. Chemical composition of culture media of banana micro-propagation in MS medium Murashige and Skooge (1962)

Constituents	Medium		
	Initiation	**Shooting**	**Rooting**
MS salt g L^{-1}	4.2	4.2	4.2
Sucrose g L^{-1}	30	30	30
BA mg L^{-1}	3.0	5.0	0.0
NAA mg L^{-1}	0.0	0.0	2.0
pH	5.8-6.0	5.8-6.0	5.8-6.0
Agar g L^{-1}	7	7	7
Culture's containers	300-mL Glass jars	300-mL Glass jars	300-mL Glass jars
Amount of media/containers	40 mL	40 mL	40 mL

MS = Murashigo and Skoog (1962). BA = 6-Benzyladenine. NAA = Naphthalene acetic acid
The pH of the medium was adjusted before autoclaving to 5.8
All media sterilized by autoclaving at 121ºC/25 min

bromide and visualized with UV transilluminator and photographed. A 50 bp DNA ladder (Promega) was used as a standard with molecular sizes of 800, 750, 700, 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100 and 50 bp. DICE computer package was used for the analysis of RAPD-PCR products (Yang & Quiros, 1993).

RESULTS AND DISCUSSION

Banana micro-propagation. Banana meristem, whitish when first sub-cultured, turned green within the first two weeks from incubation were used (Fig. 1a). Every week, it was sub-cultured onto initiation fresh medium to avoid the browning and shoot initials are formed in 2 months on initiation medium. Further differentiation and growth of shoot initials was obtained by transferring cultures onto fresh initiation medium. A small shoot was visible to the naked eye. These shoots produced multiple clusters, which were sub-cultured when the cluster transferred to multiplication medium, producing new shoots (Fig. 1b). Four to seven days after a shoot was split to induce multiplication, the outer darkened leaves and shoot base were trimmed away to reveal new side shoots and the halves were transferred to fresh medium. After 2 - 3 weeks, the new multiple shoots were separated with a scalpel and transferred again. Transfer was repeated at 3 weeks intervals since the proliferating cultures filled the vessels (Fig. 1c). Small un-rooted plantlets are transferred onto the rooting medium. This medium stimulated shoot elongation and initiated rooting of plantlets in 5 weeks (Fig. 1d).

Rooted plantlets, when reached 40 - 50 mm in height, were removed from the agar medium, washed and planted in potting mix containing a mixture of sand, peatmoss and vermiculite (1: 1: 1) in plastic pots (6 cm in width), placed in misting bed for 7 - 10 days and then transferred to normal greenhouse conditions. All plantlets survived and grew normally attaining average height of 30 cm in ten weeks. These potted plantlets are usually ready to be planted in the field in 6 to 8 weeks (Fig. 1e).

In banana, the apical meristem multiplication method obviously has great potential for producing multiple, pathogen-free planting materials in large numbers and

Fig. 1. Stages in the development of multiple shoot cultures and root formation in banana clones. (a) Meristem derived culture, (b) Growth of multiple shoots, (c) Un-rooted shoots, (d) Rooted plantlets, (e) Small plant after adaption growen in mix of sand, peatmoss and vermiculite

making germplasm preservation (Vasil & Vasil, 1980; Wang & Hu, 1980). Attempts were made to propagate some of *Musa* cultivars through excised meristems, but this required different culture media for shoot multiplication and root differentiation (Berg & Bustomante, 1974; Dore Swamy *et al*., 1983; Cronauer & Krikorian, 1984; Krikorian & Cronauer, 1984). An efficient *in vitro* clonal propagation depends on various factors, such as high frequency of multiplication, low cost and production of true-to-type plants. In bananas and plantains, traditional methods of multiplication produce 10 to 25 lateral buds (propagules) from a source plant during the course of its life cycle (Rowe & Richardson, 1985). The present findings suggested a potential of several hundred plantlets produced within a year from cultures established from a single explant. Due to

synchronous growth of plantlets regenerated *in vitro* the harvesting period was reduced to half as compared to that of conventionally propagated plants.

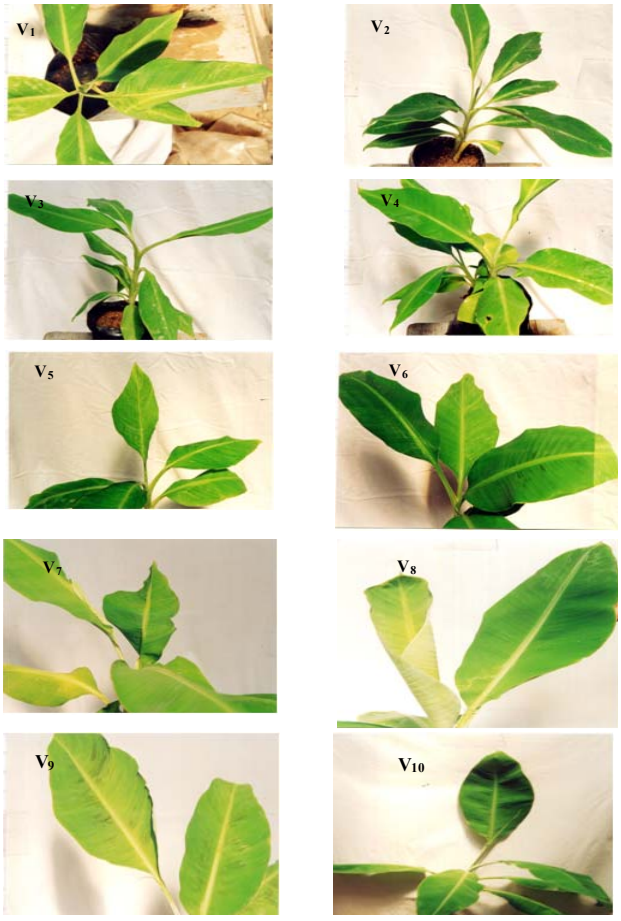
Somaclonal variation isolation. In this study, the variations were observed and recorded in both stages adaptation (Fig. 2) and nursery stage (before transplanted to the field; Fig. 3). Our results indicated that the variation in adaptation stage can be identified but the rate of occurrence is slowly. However, this rate was increased in the nursery stage, where it was easy to distinguish from the normal plant in the nursery stage. Ten off-types have been isolated and characterized depended on the morphological characterization as compared to normal plants. The following 10 types of somaclonal variants were identified of which some were comparable to those described before by Vuylsteke *et al*. (1988). All of them were characterized as modified leaf shape, or deformation as shown in Fig. 3 such as Uneven lamina (V_7), leaf becoming smaller, narrower and long but weak (V_3), leaf becoming broad and oval (V_8), leaf becoming narrow and oval (V_{10}), leaf becoming lendency to roll up at edges (V_2). Variation in the chlorophyll content of the leaf (V_5): the leaf was narrow especially at tip, resulting in a spear shape. This is usually associated with a shorter length and stains similar to burns are visible. The variegation may vary from complete absence of chlorophyll to almost normal color (V_1, V_4, V_6 & V_9).

Results indicated that all the somaclonal variation obtained were incidence, while the multiplication was carried out for sixth subculture and some of them could be observed in the adaptation stage with rate about 0.6% increased to 1.2% at nursery stage. The results of this work are in agreement with those of Stover (1986), Smith (1988) and Krikorian *et al*. (1993) showed that somaclonal variation during sub-culturing is one of the factors responsible for anomalies in micro-propagated plants. The results indicated that bio-factories should have total control of this parameter as the majority of these variants had an inferior performance in comparison with normal plants (De Langhe *et al*., 1996), as was shown in the present study. In our study, some off-type started to appear in the hardness stage (Fig. 2) and the rate of somaclonal variation after inspecting individual plants increased at the nursery stage (before transplanted to the field). Damasco *et al*. (1998) also detected variation at fifth cycle.

Shoot induction from sucker tips with BA was the most common method for *in vitro* propagation in *Musa* and the somaclonal variant index varies in a wide range between 0.1 and 60% depending on different parameters such as: genetic mosaicims, number of subculture, growth regulators concentration, donor genotype, etc. (Côte *et al*., 1993; Sandoval *et al*., 1996). Shepherd and Dos Santos (1996) have reported that the *Musa* plants regenerated through *in vitro* culture with BA show a higher percentage of mitotic abnormalities than plants, which were vegetatively propagated by classical methods. In this study, all variants

Fig. 2. Some variants isolated in adaptation stage all these variant are variegated type

Fig. 3. Some variants isolated from nursery stage. V_1 (Variegation), V_2 (Leaf becoming lendency to roll up at edges), V_3 (Leaf becoming smaller, narrower & long but weak), V_4 (Variegation), V_5 (Variation in the chlorophyll content of the leaf), V_6 (Variegation), V_7 (Un-even lamina), V_8 (Leaf becoming broad & oval), V_9 (Variegation), V_{10} (Leaf becoming narrow & oval)



obtained regenerated from adventitious shoots induced on excised shoot apices of Williams cultivar grown on MS medium (Murashige & Skoog, 1962) with 5 mg L^{-1} BA. Also, Trujillo and Garcia (1996) reported a somaclonal variant (CIEN BT A-03) resistant to yellow sigatoka (*Micosphararella musicola* Lench) regenerated from adventitious shoots induced on excised shoot apices of Williams cultivar, grown on MS medium supplemented with 15 mg L^{-1} of BA. All variants depended on the morphological characterization, either at adaptation or in nursery stage as there are limited methods to detect the off-type of banana. It means that visual screening during acclimatization in the greenhouse helps detect putative off-types, which can then be eliminated (Grillo *et al*., 1998). Multivariate analysis has been used to optimize morphological detection of off-type during acclimatization (Grillo *et al*., 1998). Smith *et al*. (1999) have detected off-type plants in ladyfinger bananas (*Musa* spp., AAB group, "pome" subgroup) during growth of plants in the greenhouse with chlorotic streaks in leaves. Hwang (1986) has reported that the percentage of off-types among 30000 - 46000 tissue-cultured plantlets derived from cavendish cv. Pei chiao was 0.37% in young plantlets and 2.43% in mature plantlets. Finally, visual screening at pre-hardening and hardening stages in greenhouse can help eliminate morphological off-type. Rouging of somaclonal variants also could be performed in such nurseries to avoid the occurrence of off-types in production field at great economic loss to the plantain or banana farmer.

Virus detection. To be sure that these variants were not generated due to virus infection, BBTV and Banana Mosaic Virus (BMV) were detected serologically (using DAS-ELISA technique). The results indicated that none of them was present in these variants. A wide range of abnormalities were caused by viruses and almost any attribute of the plant may be changed by virus infection through the most noticeable effects are changes in color such as yellowing (chlorosis), death of tissue (necrosis) and stunting of growth, but some cause abnormal growth patterns, either by preferentially restricting the development of some parts of the plant, or even by promoting additional growth. On the other hand, many virus-induced abnormalities closely resembled genetic deviations, because viruses act like genes in influencing that host via their nucleic acids. There are also a multitude of genetic abnormalities and chimaeras, which give virus-like changes in the color, distribution of pigments and growth form of plants. Grillo *et al*. (1999) reported that mass propagation of bananas through *in vitro* techniques can lead to a high percentage of somaclonal variants, one of them is mosaic-like or "Massada" type and erect variants, reminiscent of plants infected with bunchy top virus (however, negative in ELISA test) with elongated leaves compressed in a rosette at the top of the pseudostem.

Molecular characterization. Optimum conditions for DNA extraction were followed from banana leaves for reproducible PCR in the presence of three primers OPK01, OPK02 and OPK03. The results were scored as patterns of bands obtained from *in vitro* micro-propagated off-type plants compared with the normal plants (Fig. 4). Three primers produced amplification products that were monomorphic across all the off-type plants. Three oligonucleotide primers were used to amplify genomic sequences from different banana variants plants obtained through tissue culture. A number of clearly defined major fragments were amplified, often against a background of less strongly amplified minor fragments (Fig. 4). While some of these bands were common to all plants, differences between the plants were also apparent not only in the major bands but also in many of the minor fragments. OPK01 produced 92 bands rangeing from 200 bp to 1000 bp in size (Table II & Fig. 4). All variants were similar in the number and size of obtained bands except variant V_9 or normal plant, which produced two bands more than the rest of 1000 and 1200 bp. Otherwise, OPK01 produced 10 bands in case

Fig. 4. RAPD-PCR polymorphism of DNA three oligonucleotide 10 mer primes OPKO1, OPKO2 and OPKO3. V_1: V_{10} (variants), M: A 50 bp of 1kb DNA ladder (promega)

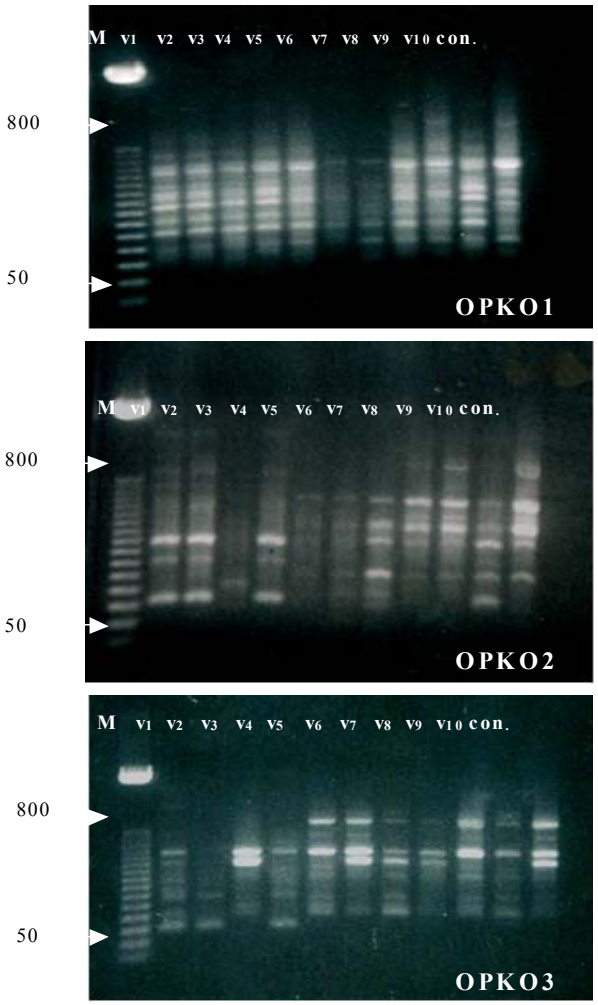


Table II. Amplified fragments obtained from the DNAs of banana micro-propagated variants *in vitro*-derived via RAPD-PCR analysis with arbitrary sequence primer tested (OPK01)

Variant	Number of total bands in the gel	Size range (bp)	Number of polymorphic bands	Size of poly-morphic bands (bp)	%of poly- morphism
V_1	18	200-750	2	1200,1400	11.1
V_2	18	200-750	2	1200,1400	11.1
V_3	18	200-750	2	1200,1400	11.1
V_4	18	200-750	2	1200,1400	11.1
V_5	18	200-750	2	1200,1400	11.1
V_6	18	200-750	2	1200,1400	11.1
V_7	18	200-750	2	1200,1400	11.1
V_8	18	200-750	2	1200,1400	11.1
V_9	20	200-1000	0	0	0
V_{10}	18	200-750	2	1200,1400	11.1

Table III. Amplified fragments obtained from the DNAs of banana micro-propagated variants *in vitro*-derived via RAPD-PCR analysis with arbitrary sequence primer tested (OPK02)

Variants	Number of total bands in the gel	Size range (bp)	Number of polymorphic bands	Size of poly-morphic bands (bp)	% of polymorphism
V_1	13	200-1600	9	1600,750,700,650	69.2
V_2	13	200-1600	9	600,550,450,400,200	69.2
V_3	9	250-500	9	1000,700,600,550	100.0
V_4	13	200-1600	9	500,450,400,300, 250	69.2
V_5	11	300-700	1	1000	9.1
V_6	12	200-700	2	1000,200	16.7
V_7	12	200-700	2	1000,200	16.7
V_8	12	300-1000	0	0	0
V_9	12	300-1000	0	0	0
V_{10}	12	200-700	2	1000,200	16.7

Table IV. Amplified fragments obtained from the DNAs of banana micropropagated variants *in vitro*-derived via RAPD-PCR analysis with arbitrary sequence primer tested (OPK03)

Variants	Number of total bands in the gel	Size range (bp)	Number of polymorphic bands	Size of polymorphic bands (bp)	% of polymorphism
V_1	17	150-1400	11	150,200,250,300,350,400, 00,550,900,950,1400	64.7
V_2	10	150-600	8	150,250,300,450,550 800,900,950	80
V_3	11	250-600	7	300,450,500,550,800 900,950	63.6
V_4	10	150-600	8	150,250,300,450,550, 800,900,950	80
V_5	15	250-900	5	300,350,500,550,950	33.3
V_6	12	250-900	2	950, 450	16.7
V_7	14	250-950	2	800, 300	14.3
V_8	11	450-900	3	950, 800, 250	27.3
V_9	14	250-900	2	950, 300	14.3
V_{10}	13	250-950	3	800, 550, 300	23.1

of type normal ranged from 250 - 1200 bp.

As shown in Table II, OPK01 produced two polymorphic bands in all variants except variant V_9, where no polymorphic band was obtained with 11.1% polymorphism, while it was 0% in case of variant V_9. OPK02 produced 65 bands ranged from 200 to 1600 bp. The number of bands ranged from three to seven bands in all variants. OPK02 produced polymorphic bands in all variants except V_8 and V_9, since no polymorphic bands obtained from OPK02 and the percentage of polymorphism is 0%. However, the percentage of polymorphism is 9.1% in V_5, but it 16.1% in V_6, V_7 and V_{10} and it increased to 69.2% in both V_1 and V_2 to 100% in V_3. It was concluded that V_1, V_2 and V_4 had same pattern of DNA profile but OPK02 showed polymorphism percentage of 69.1% compared to the normal plant. Also V_8 and V_9 have the pattern of DNA profile with no polymorphic bands compared to normal plants (Table III & Fig. 4). OPK03 produced 63 bands across in all variants and the control plants ranged for 150 bp to 1400 bp; polymorphism produced by OPK03 revealed variability in each variant. V_2 showed high polymorphism percentage (100%), but V_7 and V_9 showed low percentage of polymorphism (14.3) (Table IV & Fig. 4). The RAPD technique revealed polymorphism among the population of cv. Williams. The size of the scorable bands produced ranged from approximately 200 to 1400 bp and the number of bands ranged from 3 - 10. In general, the amount of variation differed between 10 variants. A distinct band of approximately 600 bp was consistently present in all the variants and control. The polymerase chain reaction (PCR) and the associated random amplification of polymorphic DNA (RAPD) technique in the analysis of DNA can be

used to reveal level of DNA polymorphism in regenerated banana plants. On the other hand, visual detection of off-types in micro-propagated bananas was time consuming, laborious and expensive since it is done in 3 - 4 months after field establishment (Israeli *et al*., 1991). Earlier detection of off-types in the nursery by inspecting individual plants is possible but it is again laborious and needs optimal and uniform growth conditions (Smith & Hamill, 1993).

The development and use of efficient and reliable methods for detecting off-types is of prime importance to the banana industry. Detecting off-types by molecular analysis may offer a better alternative in this regard (Damasco *et al*., 1996b). Using proteins and isozymes (Jarret & Gawel, 1995), RFLP's (Jarret & Gawel, 1995), RAPD-PCR (Kaemmer *et al*., 1992) and SCAR- PCR (Paran & Michelmore, 1993) allows off-types to be discriminated from normal plants. Detection based on morphological characteristics alone has been successful in removing off-types during nursery production, before plants are sold to growers. This has helped reestablish confidence in the use of micro-propagated planting material. The advantages of PCR-based markers over other genetic markers is advantageous since (1) PCR assay is easy to perform and requires a small amount of DNA specially when compared with the procedure involved in RFLP, (2) for RAPD, no prior sequence information is required since a number of commercially available arbitrary primers can be used and (3) the techniques are convenient to use for screening large populations. The PCR-based technique offers much potential for detecting somaclonal variants in micro-propagated bananas. RAPD analysis has been used successfully to differentiate between a gamma irradiation induced mutant of Grande Naine and its original parental line (Kaemmer *et al*., 1992). However, in Cavendish banana cultivar Valery, RAPD analysis revealed no variation between normal and off-type plants regenerated from micro-propagation (Howell *et al*., 1994).

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