MOLECULAR METHODS FOR EARLY IDENTIFICATION OF BANANA (*Musa* spp. AAA) DWARF SOMACLONAL VARIANTS

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ABSTRACT

Micropropagation offers a rapid method of producing disease-free planting materials in vegetatively reproduced species such as banana. However, widespread use of this technique is limited by the high incidence of somaclonal variation, with dwarfism as the major micropropagation induced variant (hereafter referred to as off-types) in the Cavendish subgroup (Musa spp. AAA). Dwarf off-types are rarely detected in vitro and commonly not apparent after until field planting has taken place. Identification of dwarf off-types at an early stage during in vitro culture is important. A random amplified polymorphic DNA (RAPD) marker specific to dwarf off-type was identified. Primer OPJ-04 (5'CCGAACACGG-3') consistently amplified a fragment of approximately 1,500 bp which was present in normal (true-to-type) but absent in the dwarf off-type plants of two Cavendish cultivars including New Guinea Cavendish and Williams. This RAPD fragment was cloned and a 1,625 bp nucleotide sequence was obtained. Primers homologous to this sequence were designed and used for specific polymerase chain reaction (PCR). The specific PCR offers a rapid and reliable method for identification of dwarf off-types.

INTRODUCTION

Micropropagation offers a rapid method of producing large quantities of disease-free bananas (*Musa* spp.) for commercial production. However, widespread use of such materials is hindered by the high percentages of tissue culture-induced variants (off-types), especially with Cavendish (*Musa* spp. AAA) bananas (Smith 1988; Israeli *et al.* 1991). Off-types have been noted for plant stature (mainly dwarf), leaf variegation and fruit bunch characters (small bunches, hairy fruit). Dwarfism is by far the most common off-type, accounting for more than 75% of the observed micropropagation-induced variation in the Cavendish subgroup (Israeli and Reuveni 1995). Fruit bunches produced by the dwarf off-types are of less commercial value, causing serious economic losses to growers.

Early detection and elimination of off-types from micropropagated materials is highly desirable but not easy to undertake. Dwarf off-types are not visibly detected *in vitro* and can be observed in the later stages of nursery production (i.e. two to three months after deflasking) when plants are taller than 20 cm and growing vigorously (Smith and Hamill 1993). Application of GA_3 at the deflasking stage increased the efficiency of detection, however, the GA_3 response at the *in vitro* stage did not give a clear discrimination between normal and dwarf off-type plants (Damasco *et al.* 1996). The use of polymerase chain reaction (PCR)-based marker to detect dwarf off-types following micropropagation offers several advantages over these and other detection technique such as RFLP and isozymes. The technique is rapid, results can be obtained in two days in contrast to three months for nursery evaluation or seven months for field evaluation. Only small amount of DNA is needed, such as would be available from small *in vitro* leaf. There is no requirement for greenhouse or field space since analysis of the plants can be done while they are still *in vitro*. Being DNA-based, the technique is more reliable as it is not influenced by the developmental stage of the plant or the environmental conditions under which plants are grown.

Random amplified polymorphic DNA (RAPD) analysis a PCR-based technique uses random primers to generate DNA fragments which can be used as genetic markers (Williams *et al.* 1990). Polymorphisms generated by RAPD analysis can be used for fingerprinting many plant species, genetic analysis and tagging specific traits (Michelmore *et al.* 1991; Tingey and del Tufo 1993; Howel *et al* 1994). The conversion of RAPD marker into a sequence characterised amplified region (SCAR; Paran and Michelmore 1993) allows for a fast and robust DNA assay.

This paper describes the development of a robust molecular assay based on PCR for the identification of dwarf off-types generated from banana micropropagation.

MATERIALS AND METHODS

Plant materials

All plant materials were obtained from the banana collection of the Queensland Department of Plant Industries, Maroochy Horticultural Research Station, Nambour, Queensland, Australia. Normal and dwarf off-type plants of Cavendish (*Musa* spp. AAA) cultivars New Guinea Cavendish and Williams were obtained originally from several different micropropagation events (Table 1). 'Dwarf Parfitt', a naturally occurring extra-dwarf Cavendish cultivar, was also included in the study to determine if the dwarf mutation generated from micropropagation is the same as that occurring in somatic mutants. Suckers harvested from these plants were cultured *in vitro* following the procedure of Drew and Smith (1990). *In vitro*- and glasshouse-grown (New Guinea Cavendish accession C420, Williams accessions C76 and C117, and Dwarf Parfitt) and field-grown (New Guinea Cavendish accessions C75 and C529 and Williams accessions C251, C271 and C117) normal and dwarf off-type plants were analyzed using RAPD. The accession number (e.g. C117) represents the original clonal plant from which the original tissue cultures were initiated. In total, 116 (57 normals, 59 dwarfs) plants were used for RAPD analysis.

Table 1. Normal (N) and dwarf off-type (D) plants of Cavendish cultivars New Guinea Cavendish (NGC), Williams and Dwarf Parfitt (DP) used in RAPD analysis. All plant materials were obtained from QDPI Maroochy Horticultural Research Station, Nambour, Australia. Each accession represents at least one independent tissue culture event. Plants from sucker were regenerated through axillary and/or adventitious budding while plants from floral apex tissue were regenerated from callus through adventitious budding. The different accessions were collected from the South Johnstone Research Station, South Johnstone (SJRS), Redland Bay (RB), Cleveland (C), Mena Creek (MC), Kennedy (K), and Wamuran (W), Queensland, Australia.

Cultivar	Plant type	Accession number	Number of plants tested			Initial explant	Place of collection
			In vitro	Glasshouse	Field		
NGC	N	C420	14	7	–	sucker	SJRS
	D	C420	14	5	–	sucker	SJRS
NGC	N	C75	–	–	4	floral apex	RB
	D	C75	–	–	5	floral apex	RB
Williams	N	C76	5	5	–	floral apex	SJRS
	D	C76	5	5	–	floral apex	SJRS
Williams	N	C251	–	–	7	sucker	MC
	D	C251	–	–	2	sucker	MC
Williams	N	C117	8	3	4	sucker	K
	D	C117	9	3	3	sucker	K
DP	D	M503	2	2	1	sucker	W
Total			57	30	29	116	

DNA extraction

Total genomic DNA was extracted from fresh leaves of *in vitro*-, glasshouse- and field-grown plants using a modified CTAB (hexadecyltrimethylammonium bromide) method (Graham *et al.*, 1994). Leaf tissues (1 to 2 g) were frozen in liquid nitrogen and ground in a mortar and pestle. For some *in vitro* plants with small leaves (<0.2 g), leaf tissues were frozen and ground in a microfuge tube (1.5 mL) using a micropestle. The DNA pellet was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and yield quantified using a Beckman Du-64 Spectrophotometer equipped with a soft pac module for nucleic acid determination (Beckman Instruments Inc., Fullerton, CA, USA).

DNA amplification and electrophoresis

RAPD reactions were performed in volumes of 25 µL containing 1 x reaction buffer (10mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mMKCl), 2.5 to 4.0 mM $MgCl_2$ (depending on primer), 400 µM each of dATP, dCTP and dGTP and dTTP (Promega, Madison, WI, USA), 0.25 µM of arbitrary decamer primer (Operon Technologies, Alameda, CA, USA), 50 ng of banana genomic DNA, 1.0 unit of *Taq* DNA polymerase (Boehringer Mannheim, Mannheim, Germany) and overlaid with a drop of paraffin oil. Amplification reactions were performed in a 480 Perkin Elmer DNA thermocycler (Perkin Elmer Corp., Norwalk, CT, USA). An initial denaturation temperature of 94°C for 5 min was followed by 45 cycles each at 94°C for 1 min, 37°C for 1 min and 72°C for 2 min. For specific PCR, DNA amplification reactions were performed in volumes of 20 µL containing 1 x reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg mL^{-1} gelatin), 200 µM each of dATP, dCTP, dGTP and dTTP, 3.0 mM $MgCl_2$ (including that in the reaction buffer), 0.1 µM primer, 1 unit of *Taq* DNA polymerase and 50 ng of banana genomic DNA. Amplification reactions were performed in a PT-100-96 Thermal Controller (Bresatec Pty. Ltd., Thebarton, SA, Australia). An initial denaturation at 94°C for 3 min was followed by 35 cycles each at 94°C for 10 s, 50 to 65°C (depending on primers) for 10 s and 72°C for 1 min. The amplification products were analyzed on a 1.2% agarose (Promega, Madison, WI, USA) gel in 1 x TBE buffer containing ethidium bromide (0.25 µg mL^{-1}). The amplification products were visualized under UV light (302 mm) and photographed using 667 polaroid film (Polaroid (UK) Ltd., Hertfordshire, England).

Identification of polymorphisms between normal and dwarf off-type plants

Initially, the usefulness of RAPD analysis in detecting polymorphisms between normal and dwarf off-type bananas was determined using *in vitro*-grown New Guinea Cavendish accession C420. Sixty-six arbitrary decamer primers from kits A (OPA-01, 04, 06, 07, 09, 10, 12, 13, 18-20), H (OPH-01-20), J (OPJ-01-15), S (OPS-01-05) and U (OPU-01-15) were used. Five *in vitro* normal and 5 *in vitro* dwarf off-type plants were tested for each primer. The primers which initially

revealed polymorphisms between normal and dwarf off-type plants were further evaluated for markers specific to the dwarf off-type plants using 81 micropropagated plants from New Guinea Cavendish (20 normals, 21 dwarf off-types) and Williams (22 normals, 18 dwarf off-types). The RAPD marker identified as being specific to the dwarf off-types was further tested on Williams accession C76 (10 normals, 10 dwarf off-types) and Dwarf Parfitt (5 plants).

Cloning and sequencing of the RAPD marker band

The polymorphic RAPD marker band was excised from an ethidium bromide stained low melting temperature agarose gel (2.0%) under UV illumination. The DNA was extracted from the agarose gel slice using phenol following the procedure of Sambrook *et al.* (1989). The purified RAPD marker band was cloned using the TA cloning system (Invitrogen Corp., San Diego, CA, USA) following the manufacturer's instructions.

DNA sequencing was performed using the Prism™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing (Applied Biosystems Inc., Foster City, CA, USA) according to the "Protocol for Cycle Sequencing" supplied by the manufacturer. M13 forward and reverse primers were used to sequence the insert from both ends and the remaining sequences were obtained by progressively moving the priming site along the DNA sequence (Sambrook *et al.* 1989). Oligonucleotide primers were synthesized by the Oligonucleotide Synthesis Facility, Centre for Molecular Biology, The University of Queensland, Brisbane, Australia. The sequences generated were analysed by the computer program SeqEd™ (Version 1.0.3; Applied Bio Systems Inc.).

SCAR analysis

Three pairs of primers were designed and synthesized to be used as dwarf off-type SCAR primers (Table 2). The first pair contained the RAPD primer sequences plus the next 10 internal bases from both ends of the RAPD marker sequence. The other two pairs were the primers used in the sequencing of the RAPD marker band. The 3 pairs of SCAR primers were tested on 12 normal and 12 dwarf off-type plants previously analyzed by RAPD (Table 1).

Specific PCR for detecting dwarf off-types

The effectiveness of the specific PCR technique to detect dwarf off-type plants while still at the *in vitro* stage was tested in collaboration with a commercial tissue culture laboratory. A random sample of 158 *in vitro* plantlets of Williams (accession C724) was taken at subculture cycle 4 of the multiplication stage and DNA extracted. Amplification reactions were performed using a standard reaction mixture, 2 µL of unquantified DNA, SCAR primer pair B_1B_2 and an annealing temperature of 50°C. Plants were assayed for the presence (normal) or absence (dwarf) of the $SCAR_{1343}$ band.

Table 2. Sequence of the 20-mer specific oligonucleotide primers derived from the RAPD marker and the expected size of SCAR band. The underlined sequences in primers A_1 and A_2 represent the sequence of the OPJ-04 RAPD primer. Primers B_1, B_2 and C_1 were used in sequencing the RAPD marker band.

Primer	*Primer Sequence (5' to 3')*	*Expected size of SCAR band (bp)*
A_1	CCGAACACGGGACTTATACA	1,625
A_2	CCGAACACGGGCTAACCTAG	
B_1	CTGTGGTTGCATTCTCATAC	1,343
B_2	GTGAATCATACTCGCGAACC	
C_1	TTAAGCCTTGGATTGACTGC	994
B_2	GTGAATCATACTCGCGAACC	

RESULTS

Polymorphisms in New Guinea Cavendish and identification of dwarf off-type specific marker

The initial 66-primer screen amplified a total of 234 products with between 1 and 10 products generated per primer. Nineteen of the 66 primers (28.8%) revealed polymorphisms between normal and dwarf off-type plants, with 34 polymorphic products generated. Three primers (OPJ-02, OPJ-08, OPU-04) did not amplify any products. The DNA and $MgCl_2$ concentrations in the reaction mixture were important for successful DNA amplifications in banana. Using 50 ng of banana genomic DNA as template and 2.5 to 4.0 mM $MgCl_2$ (depending on primer), field-, glasshouse- or *in vitro*-grown plants of a particular cultivar and/or accession produced the same scorable banding pattern.

The profiles of amplified products from New Guinea Cavendish (accessions C420, C75 and C529), and Williams (accessions C271, C251 and C117) and their associated micropropagation-produced dwarf off-types were compared for identification of markers specific to dwarf off-types. Of the 19 primers which revealed polymorphisms in the initial primer screen using normal New Guinea Cavendish accession C420 and its respective dwarf off-type, only 10 primers revealed polymorphisms for New Guinea Cavendish accessions C75 and C529 and its respective dwarf off-type, 7 primers for Williams accessions C251 and C271 and its respective dwarf off-type, and 4 primers for Williams accession C117 and its respective dwarf off-type (Table 3). The majority of the polymorphisms generated were not associated with dwarfism. Six primers (OPA-06, OPH-13, OPJ-04, OPJ-10, OPJ-13, OPU-06) were able to differentiate between normal and dwarf New Guinea

Cavendish (3 accessions), and 2 primers (OPJ-04, OPJ-13) differentiated between normal and dwarf Williams (2 accessions; Table 4). Of the DNA bands which differentiated normal and dwarf off-type plants of different accessions, only the 1,500 bp band from primer OPJ-04 (referred to as OPJ-04_{1500}) was found consistently present in all normal but absent in all dwarf off-type plans of New Guinea Cavendish (3 accessions) and Williams (2 accessions; Table 4, Figure 1). This primer was further tested on normal and dwarf Williams accession C76. The OPJ-04_{1500} band was consistently present in all normal but absent in all dwarf off-type plants. The naturally occurring extra dwarf cultivar, Dwarf Parfitt, gave an OPJ-04 RAPD profile similar to that of normal plants of Williams and New Guinea Cavendish (Figure 1), and the OPJ-04_{1500} band was present in all Dwarf Parfitt plants tested.

DNA Sequencing, primer construction and SCAR amplification

Sequencing of the dwarf off-type RAPD marker band generated 1,625 bp sequence. The terminal 10 bases exactly matched the OPJ-04 primer sequences. Three pairs of 20-mer SCAR primers were synthesized and tested using genomic DNA from normal and dwarf New Guinea Cavendish and Williams. In all normal plants, a 1,625 bp band was amplified using SCAR primer pair A_1A_2 (Figure 2A), a band 1,343 bp was amplified using SCAR primer pairs B_1B_2 (Figure 2B), and a band of 944 bp was amplified by primer pair C_1B_2 (Figure 2C). The SCAR bands were not amplified from the dwarf off-type DNA. The RAPD polymorphism was retained as the presence or absence of the band with the corresponding SCAR primers. All the three pairs of SCAR primers tested showed SCAR amplification only in normal plants and not in the dwarf off-types and therefore either of the three primer pairs could be used for detecting dwarf off-type plants. Thus, in all *in vitro* detection experiments, only the SCAR pair B_1B_2 was used in assaying for normal and dwarf off-type plants.

Apart from the major band, sometimes less intense bands were also amplified in both normal and dwarf off-type plants using the SCAR primers. Elevating the annealing temperature from 50°C to 65°C prevented the amplification of minor bands but did not affect the amplification of the SCAR band.

***In vitro* detection of dwarf off-types using specific PCR**

Of the 158 plantlets analysed from the commercial micropropagation system (Figure 3), 10 plantlets showed no amplification of the SCAR 1343 band, indicating that those plantlets were likely to be dwarfs. Those 10 plants showing the absence of amplification were then assessed by RAPD analysis and confined to be lacking in the OPJ-04_{1625} band.

Table 3. Number of amplification and polymorphic products from New Guinea Cavendish (NGC, 3 accessions) and Williams (3 accessions) obtained with 19 arbitrary primers. Each accession represents at least one independent tissue culture event.

Primer	Sequence 5' to 3'	Cultivar and accession number							
		NGC (C420)		NGC (C75, C529)		Williams (C271, C251)		Williams (C117)	
		No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products
OPA-06	GGTCCCT	6	2	6	1	6	0	7	1
OPA-12	TCGGCG	5	1	5	0	5	0	6	1
OPA-13	CAGCAC	10	1	10	1	10	0	10	0
OPA-19	CAAACG	6	1	6	0	6	0	6	0
OPH-05	AGTCGT	5	3	5	0	5	0	5	0
OPH-06	ACGCAT	3	1	3	0	3	0	3	0
OPH-09	TGTAGCT	7	1	7	1	7	0	7	0
OPH-13	GACGCC	6	3	6	1	5	1	5	0
OPH-17	CACTCTC	7	1	7	1	7	0	7	0
OPH-19	CTGACC	6	1	8	0	8	0	8	0
OPJ-04	CCGAAC	7	2	7	1	7	1	7	1
OPJ-10	AAGCCC	6	2	6	1	6	1	6	0
OPJ-13	CCACACT	5	1	5	0	6	1	6	1
OPS-03	CAGAGG	5	3	5	0	5	2	5	0
OPS-04	CACCCCC	6	4	6	0	6	0	6	0

Table 3. (continued)

Primer	Sequence 5' to 3'	Cultivar and accession number							
		NGC (C420)		NGC (C75, C529)		Williams (C271, C251)		Williams (C117)	
		No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products	No. of amplification products	Polymorphic products
OPU-06	ACCTTTG	7	1	7	1	7	1	6	0
OPU-07	CCTGCTC	7	3	7	1	7	1	7	0
OPU-08	GGCGAA	7	1	7	1	7	0	7	0
OPU-15	ACGGGC	5	2	5	0	5	0	5	0

Table 4. Primers that revealed polymorphisms between normal and dwarf off-type plants of New Guinea Cavendish (NGC, 3 accessions) and Williams (2 accessions). The (+) indicates presence and (–) absence of amplification. Each accession represents at least one independent tissue culture event.

Primer	Approx fragment size (bp)	Cultivar, accession number and stature							
		NGC (C420)		NGC (C529,C75)		Williams (C251)		Williams (C117)	
		Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf
OPA-06	600	–	+	–	+	+	+	+	+
	1,300	–	+	–	–	–	–	–	+
OPH-13	1,400	+	–	+	–	–	–	–	–
	1,300	–	+	+	+	–	+	+	+
OPJ-04	1,500	+	–	+	–	+	–	+	–
OPJ-10	1,300	–	+	–	+	+	+	+	+
	1,400	+	+	+	+	+	–	+	+
OPJ-13	1,300	–	+	–	+	+	+	+	+
	700	+	+	+	+	+	–	+	–
OPU-06	700	–	+	–	+	+	–	–	–
OPU-08	800	–	+	+	+	+	–	+	+

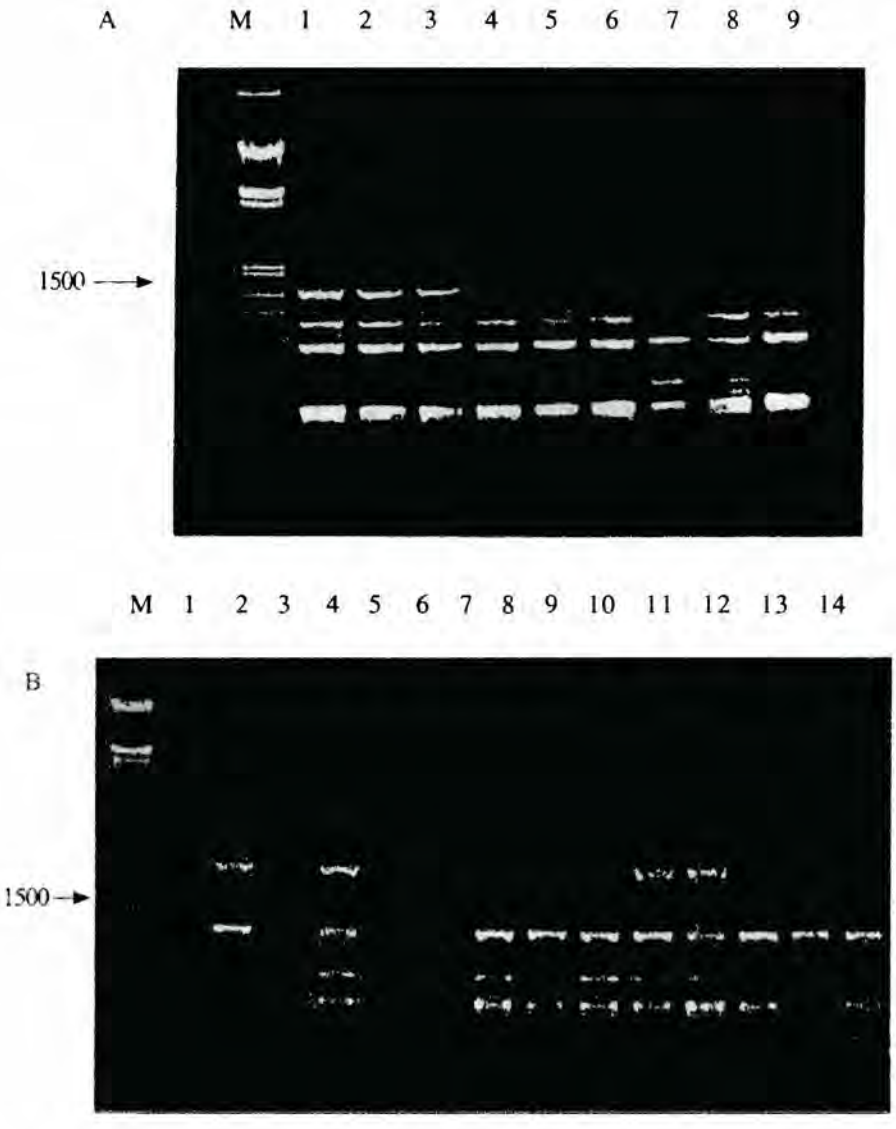

Figure 1. RAPD profiles of Cavendish cultivars obtained with primer (OPJ-04: (A) lanes 1-3 New Guinea Cavendish accession C420 normal plants, 4-8 Dwarf Parfitt, (b) lanes 1-6 Williams accession C251 normal plants, 7-9 dwarf off-types, 10-11 Williams accession C117 normals, 12-14 dwarf off-types, M-molecular weight marker, lanbda DNA cut with EcoRI and HindIII (Progen Industries Ltd., Qld, Australia). Arrow indicates the $OPJ\text{-}04_{1500}$ band.

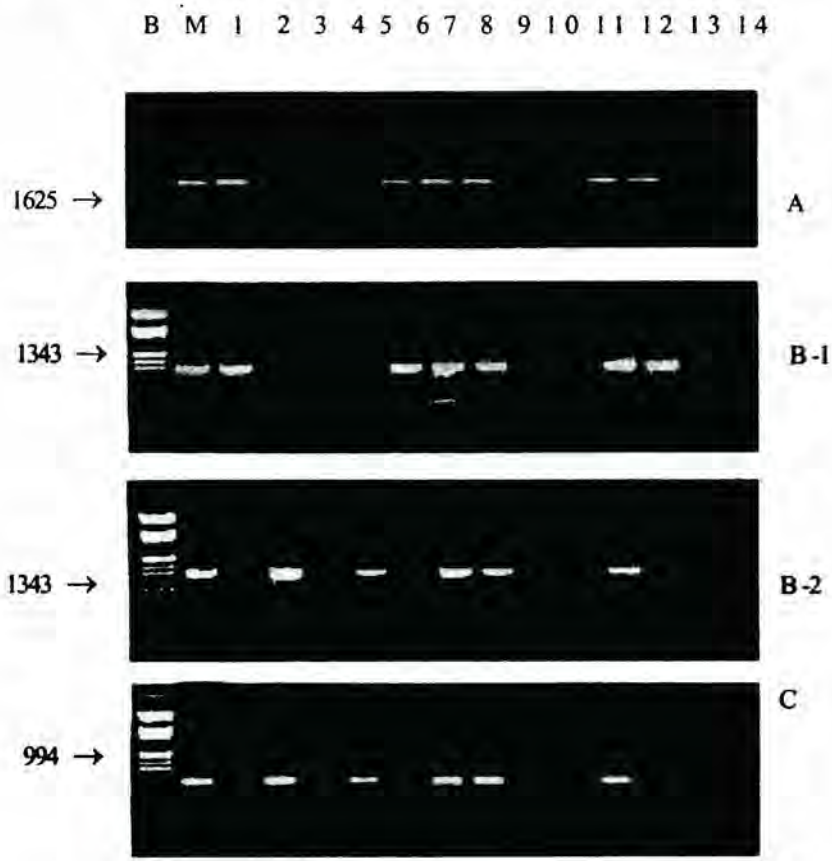

Figure 2. Amplification of SCAR in New Guinea Cavendish (NGC) and Williams. A amplification of $SCAR_{1625}$ using primer pair A_1A_2 and annealing temperature of 65°C. Lanes 1-2 normal, 3-5 dwarf (NGC accession C75, field grown plants), 6-8 normal, 9-10 dwarf (Williams accession C251, field grown plants), 11-12 normal, 13-14 normal, 13-14 dwarf (Williams accession C117, field grown plants). B amplification of $SCAR_{1343}$ using primer pair B_1B_2 and annealing temperature of 50°C. B-1 lanes 1-14 the same as in A, B-2 lanes 1 normal, 2 dwarf (NGC accession C420, in vitro plants), 3 normal, 4 dwarf (Williams accession C117, in vitro), 5 normal, 6 dwarf (Williams accession C76, in vitro), 7-8 normal, 9-10 dwarf (NGC accession C420, glasshouse), 11 normal, 12-14dwarf (Williams accession C117, glasshouse). C amplification of $SCAR_{994}$ using primer pair C_1B_2 and annealing temperature of 50°C. Lanes 1-14 the same as in B-2. M-molecular weight marker, lambda DNA cut with EcoRI and HindIII. Arrow indicates the SCAR 1625, 1343 and 994 band.

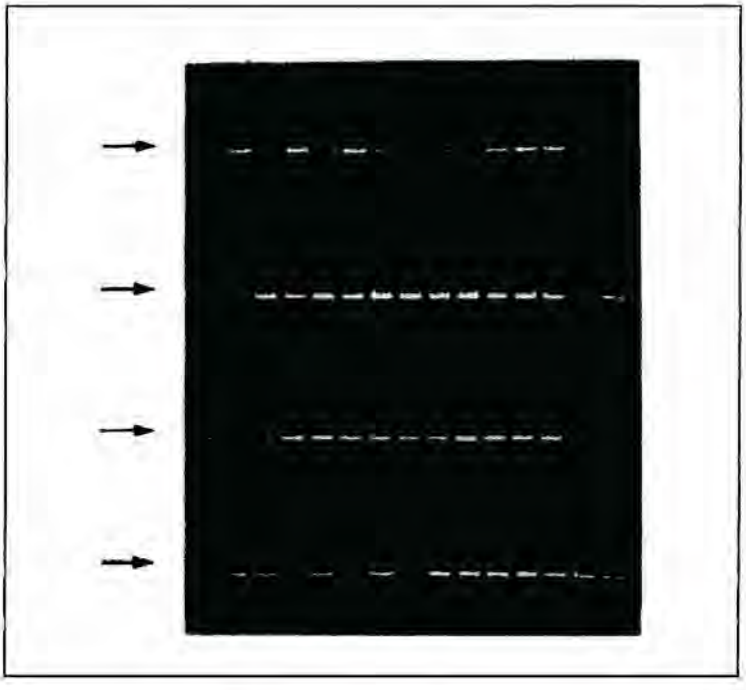

Figure 3. Assay for the presence (+) and absence (–) of $SCAR_{1343}$ band in micropropagated plants. The presence of band indicates normal plant and absence dwarf off-type. Arrow indicates the $SCAR_{1343}$ band. Amplification reactions were performed using primers B_1B_2, 2 mL of unquantified template DNA obtained from a PCR mini prep extraction and 50°C annealing temperature. The amplification products were analysed by electrophoresis on 1.2% agarose gel run for 1 h at 100 V. The first lane on top is the molecular weight marker (lambda DNA cut with EcoRI and HindIII). The assay was performed on in vitro plants of Williams (accession C724) samples at subculture cycle 4 of the multiplication stage.

DISCUSSION

A RAPD marker specific to the dwarf off-type plants from micropropagation was identified after an analysis of 57 normal and 59 dwarf off-type plants generated from several different micropropagation events. The RAPD band, $OPJ\text{-}04_{1500}$, was consistently present in all normal and absent in all dwarf off-types for all accessions of New Guinea Cavendish and Williams banana cultivars. Furthermore, the band was absent in all dwarf off-types tested regardless of initial explant source (sucker or floral apex) or mode of shoot regeneration (axillary or adventitious budding). The results therefore suggest that only one type of dwarf mutation exists in all of these micropropagated plants.

Other DNA polymorphisms observed in micropropagated bananas were not associated with the dwarf trait and could be due to other somaclonal changes during micropropagation. The polymorphism observed with the Dwarf Partfitt (a naturally occurring extra-dwarf) and micropropagation-induced dwarf off-types from New Guinea Cavendish and Williams suggests that different dwarf mutations must have occurred and that the dwarf trait marker seems to be associated with dwarfism in micropropagated Cavendish bananas only.

The conversion of the dwarf off-type specific RAPD marker into a dominant SCAR marker allowed for the development of a more rapid and robust PCR-based detection technique for dwarf off-types. All pairs of specific oligonucleotide primers resulted in the amplification of a single major band of expected size based on the sequence of the RAPD marker. The amplification of the specific band occurred only in normal plants and not in any of the dwarf off-type plants of New Guinea and Williams. For the first time, this PCR marker presents the opportunity to reliably identify all dwarf off-type plants *in vitro*, a situation not achievable based on morphology (Smith and Hamill 1993), even with the aid of GA_3 application (Damasco *et al.* 1996).

In other crops, the conversion of RAPD into PCR markers gave a more reproducible assay than could be obtained with RAPD analysis (Paran and Michelmore 1993; Francis *et al.* 1995; Yang and Korban 1996). This would facilitate the use of molecular markers for other applications such as in marker-aided selection, fingerprinting and in molecular taxonomy (Paran and Michelmore 1993; Yang and Korban 1996). In banana, the use of the dwarf off-type specific SCAR marker of the *in vitro* stage affords a reliable means for early detection of dwarf off-types allowing for their elimination before planting of micropropagated plants in the field. In addition, this SCAR marker could be particularly useful for testing the genetic integrity of banana *in vitro* stock materials for micropropagation. Those shoots with a normal plant banding pattern could be multiplied further while those showing the dwarf off-type banding pattern could be discarded. The use of proliferated shoots with a normal banding pattern for further multiplication would reduce the cost of micropropagating bananas as compared to initiating multiplication from new dwarf off-types. Further, micropagation of these normal

shoots, however, could also result in production of new dwarf off-types. This dwarf off-type SCAR marker will be a useful tool in characterising the nature of the somaclonal variation mechanism that has produced the dwarf. Of particular interest is pinpointing the locus of this genomic alteration. Moreover, this marker can be used in determining whether the polymorphism is pre-existing and enriched by the micropropagation technique, or if the genomic alteration is actually induced in culture.

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