IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Detection of Somaclonal Variations in Banana CV. Grand Naine (AAA) Using Random Amplified Polymorphic DNA (RAPD) Technique

Prathibha K. Y.[1], Keshamma E.[2], Kavitha K. R.[3]

[1]Associate Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[3]Associate Professor, Department of Botany & PG Studies, Nrupathunga University, Bengaluru, Karnataka, India

Abstract: Current research investigation aimed to detect variations of somaclones in the banana cv. Grand Naine by using random amplified polymorphic DNA (RAPD) molecular marker technique. Matured leaves that are free from damage caused by pests or disease were collected in brown covers from different field places of Bengaluru, Karnataka. Isolation of genomic DNA was carried out by cetyl trimethyl ammonium bromide. The integrity and purity of DNA and visualization of amplified products was done by electrophoresis and quantified. PCR amplification of DNA from banana somaclones using RAPD, markers were carried out using Taq DNA polymerase and short stretch of oligonucleotides (primers), which are specific to the DNA to be amplified. Then the RAPD, markers are used to assess the genetic variation in somaclonal variants of banana cv. Grand Naine. The potential of molecular markers have been used for testing the genetic fidelity of micropropagated cv. Grand Naine (AAA) plants in the fields. Molecular markers like RAPD used to identify the genetic variations that has resulted in banana cv. Grand Naine. All the markers tried clearly showed polymorphism between the off types and the normal banana plants. In conclusion, 9-17% polymorphism was observed in variants indicating the level of genetic change. The primers OPS-05 was redesigned as a marker for dwarf off-type and AR-14 for dwarf non-flowering.

Keyword: RADP, OPS-5, AR-14, Molecular markers, Polymorphism

1. INTRODUCTION

Somaclonal variation is the genetic variation that is observed when plants are regenerated from cultured somatic cells [1]. During micropropagation of bananas and plantains, somaclonal variation can occur in regenerated plants. This variation may interfere with the use of these cultures in the commercial multiplication and in the production of stable lines for genetic transformation or physical or chemical mutagenesis. Somaclonal variation is not limited to any particular group of plants. It has been reported in ornamentals, vegetable and food crops, forest species, fruit, plantation crops. The economic consequences of somaclonal variation can be enormous for farmers who use tissue cultured plants in the field. It has been observed that extreme variability of tissue cultured banana plants in terms of yield, quality etc., resulting in heavy loss to the farmers. This has also resulted in legal dispute between the farmers and the companies that supplied the tissue culture plant material. The use shoot tip culture for banana micropropagation, conservation and exchange of germplasm may be reduced by the occurrence of undesired somaclonal variants at high percentages. The off-types have delayed widespread industry acceptance of micropropagated banana [2]. It is becoming increasingly clear that somaclonal variation is usually undesirable.

Very little is known about the causes of somaclonal variation in bananas [3]. Novak explained the possible origin of somaclonal variation in Musa tissue cultures by three mechanisms: a) Variation is already present in original explant b) Variation is a result of stress induced by the conditions of the tissue culture environment; and c) Variation is induced by the specific mutagenic action of the tissue culture media [4]. In Musa there are several convincing reports of the incidence of somaclonal variation at morphological, cytological (chromosome number, structure) cytochemical (genome size), biochemical (proteins and isozymes) and molecular (nuclear genomes) [5-8]. Various range of variation are detected at various experimental sites with different varieties in banana from zero to as high as 93.4% [9]. Uma et al. reported traits affected by somaclonal variation in banana like dwarfism, sick looking tall plants, abnormal foliage, bunch, fingers, colour changes in pseudostem, petiole, bract, warty fruits, etc., [10]. The extent of variation depends on a number of factors like ploidy level, culture media etc...[11].

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Clonal propagation of horticultural species such as banana, oil palm is intended to produce chosen elite individuals in mass. Even a low percentage of somaclonal off-types is unacceptable for commercial use [12]. A practical means is to minimize somaclonal variations that can be detected in the nursery. Unfortunately, many off-types can only be recognized at a later stage in the field [13]. Hence effective somaclonal screening is the requirement for the present crop improvement of banana. Reports detecting somaclones using molecular techniques are available in other cvs [8,14]. Hence, the present study was designed with the main purpose of detection of variations of somaclones in the banana cv. Grand Naine by using random amplified polymorphic DNA (RAPD) molecular marker study.

2. MATERIALS AND METHODS

2.1 Source of planting material

Matured leaves that are free from damage caused by pests or disease were collected in brown covers from different fields places like Anekal, Kadur, Hulimavu, Hesaraghatta. Source of these micropropagated planting materials were from Khodays Biotech, MSR Biotech, Green earth, Ramco Biotech companies.

2.2 Isolation of genomic DNA by CTAB method (cetyltrimethyl ammonium bromide)

This is an efficient method for isolating plant genomic DNA from leaf tissues. It provides high quality preparation of high molecular DNA. CTAB is used to liberate the nucleic acid from cell which was further purified by phenol-chloroform to remove proteins and other contaminating plant debris. The protocol for isolation of genomic DNA was carried according to Porebski et al. 1997 [15].

2.3 PCR amplication of DNA

Polymerase chain reaction is a very simple method for in-vitro amplification of specific nucleic acids using Taq DNA polymerase and short stretch of oligonucleotides (primers), which are specific to the DNA to be amplified. It is carried out in three steps at discrete temperatures. a) Heat denaturation of template DNA at 94-98oC, b) Annealing of primers to template DNA at 35-55oC and c) Primer extension from their 3' ends at 72oC. These steps are repeated as cycles (30 to 40). The length of the product generated during the PCR is equal to the sum of the length of the primers plus the distance in the target sequence [16].

2.4 RAPD to detect genetic variation in micropropagated banana CV. Grand naine

RAPD requires very little material for analysis and relatively insensitive to the integrity of the material. Single strands of DNA of known sequence (primers) of 10 base pair long are used to prime the polymerase reaction with Taq polymerase, dNTPS to generate RAPDs. These amplified products are resolved on agarose gel and visualized under ultraviolet light. RAPD markers have been used for identification of somaclonal variation in Musa cvs. In the present study 150 primers have been used to detect variation in somaclones and normal plants of banana cv. Grand Naine. RAPD is very fast way of obtaining information about genetic variability. The list and the sequence of the primer used is given in the Table 1. Image J software were used to generate density graph plots between true-to-type and dwarf off-types of banana cv. Grand Naine [17].

Table. 1. RAPD Primer Sequences Used for Screening

S. No.	Name of Primers	Sequence of the Primers	S. No.	Name of Primers	Sequence of the Primers
01	OPA 02	TGCCGAGCTG	31.	OPD 08	GTGTGCCCCA
02	OPA 09	GGGTAACGCC	32.	OPD 09	CTCTGGAGAC
03	OPB 17	AGGGAACGAG	33.	OPD 10	GGTCTACACC
04	OPC 01	TTCGAGCCAG	34.	OPD 11	AGCGCCATTG
05	OPC 02	GTGAGGCGTC	35.	OPD 12	CACCGTATCC
06	OPC 03	GGGGGTCTTT	36.	OPD 13	GGGGTGACGA
07	OPC 04	CCGCATCTAC	37.	OPD 14	CTTCCCCAAG
08	OPC 05	GATGACCGCC	38.	OPD 15	CATCCGTGCT
09	OPC 06	GAACGGACTC	39.	OPD 16	AGGGCGTAAG
10	OPC 07	GTCCCGACGA	40.	OPD 17	TTTCCCACGG
11	DPC 08	TGGACCGGTG	41.	OPD 18	GAGAGCCAAC
12	OPC 09	CTCACCGTCC	42.	OPD 19	CTGGGGACTT

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

13	OPC 10	TGTCTGGGTG	43.	OPD 20	ACCCGGTCAC
14	OPC 11	AAAGCTGCGC	44.	OPE 11	GAGTCTCAGG
15	OPC 12	TGTCATCCCC	45.	OPE 12	TTATCGCCCC
16	OPC 13	AAGCCTCGTC	46.	OPE 14	TGCGGCTGAG
17	OPC 14	TGCGTGCTTG	47.	OPE 15	ACGCACAACC
18	OPC 15	GACGGATCAG	48.	OPE 16	GGTGACTGTG
19	OPC 16	CACACTCCAG	49.	OPE 17	CTACTGCCGT
20	OPC 17	TTCCCCCCAG	50.	OPE 19	ACGGCGTATG
21	OPC 18	TGAGTGGGTG	51.	OPE 20	AACGGTGACC
22	OPC 19	GTTGCCAGCC	52.	OPF 01	ACGGATCCTG
23	OPC 20	ACTTCGCCAC	53.	OPF 11	TTGGTACCCC
24	OPD 01	ACCGCGAAGG	54.	OPF 12	ACGGTACCAG
25	OPD 02	GGACCCAACC	55.	OPF 13	GGCTGCAGAA
26	OPD 03	GTCGCCGTCA	56.	OPF 14	TGCTGCAGGT
27	OPD 04	TCTGGTGAGG	57.	OPF 15	CCAGTACTCC
28	OPD 05	TGAGCGGACA	58.	OPF 16	GGAGTACTGG
29	OPD 06	ACCTGAACGG	59.	OPF 17	AACCCGGGAA
30	OPD 07	TTGGCACGGG	60.	OPF 18	TTCCCGGGTT
61	OPF 19	CCTCTAGACC	92.	OPK 14	CCCGCTACAC
62	OPF 20	GGTCTAGAGG	93.	OPK 15	CTCCTGCCAA
63	OPG 02	GGCACTGAGG	94.	OPK 16	GAGCGTCGAA
64	OPG 04	AGCGTGTCTG	95.	OPK 17	CCCAGCTGTG
65	OPG 05	CTGAGCGGA	96.	OPK 18	CCTAGTCGAG
66	OPG 06	GTGCCTAACC	97.	OPK 19	CACAGGCGGA
67	OPG 07	GAACCTGCGG	98.	OPK 20	GTGTCGCGAG
68	OPG 08	TCACGTCCAC	99.	OPS 01	CTACTGCGCT
69	OPG 09	CTGACGTCAC	100	OPS 02	CCTCTGACTG
70	OPG 10	AGGGCCGTCT	101	OPS 03	CAGAGGTCCC
71	OPG 15	ACTGGGACTC	102	OPS 04	CACCCCCTTG
72	OPI 16	TCTCCGCCCT	103	OPS 05	TTTGGGCCT
73	OPI 17	GGTGGTGATG	104	OPS 06	GATACCTCGG
74	OPI 18	TGCCCAGCCT	105	OPS 07	TCCGATGCTG
75	OPI 19	AATGCGGGAG	106	OPS 08	TTCAGGGTGG
76	OPI 20	AAAGTGCGGG	108	OPS 09	TCCTGGTCCC
77	OPJ 01	CCCGGCATAA	110	OPS 10	ACCGTTCCAG
78	OPJ 04	CCGAACACGG	111	OPS 11	AGTCGGGTGG
79	OPK 01	CATTCGAGCC	112	OPS 12	CTGGGTGAGT
80	OPK 02	GTCTCCGCAA	113	OPS 13	GTCGTTCCTG
81	OPK 03	CCAGCTTAGG	114	OPS 14	AAAGGGGTCC

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

82	OPK 04	CCGCCCAAAC	115	OPS 15	CAGTTCACGG
83	OPK 05	TCTGTCGAGG	116	OPS 16	AGGGGGTTCC
84	OPK 06	CACCTTTCCC	117	OPS 17	TGGGGACCAC
85	OPK 07	AGCGAGCAAG	118	OPS 18	CTGGCGAACT
86	OPK 08	GAACACTGGG	119	OPS 19	GAGTCAGCAG
87	OPK 09	CCCTACCGAC	120	OPS 20	TCTGGACGGA
88	OPK 10	GTGCAACGTG	121	OPAG01	CTACGGCTTC
89	OPK 11	AATGCCCCAG	122	OPAG02	CTGAGGTCCT
90	OPK 12	TGGCCCTCAC	123	OPAG03	TGCGGGAGTG
91	OPK 13	GGTTGTACCC	124	OPAG04	GGAGCGTACT
125	OPAG05	CCCACTAGAC	153	OPAR13	GGGTCGGCTT
126	OPAG06	GGTGGCCAAG	154	OPAR14	CTCACAGCAC
127	OPAG07	CACAGACCTG	155	OPAR15	ACACTCTGCC
128	OPAG08	AAGAGCCCTC	156	OPAR16	CCTTGCGCCT
129	OPAG09	CCGAGGGGTT	157	OPAR17	CCACCACGAG
130	OPAG10	ACTGCCCGAC	158	OPAR18	CTACCGGCAC
131	OPAG11	TTACGGTGGG	159	OPAR19	CTGATCGCGG
132	OPAG12	CTCCCAGGGT	160	OPAR20	TGCGCCATCC
133	OPAG13	GGCTTGGCGA	161	OPBG01	GTGGCTCTCC
134	OPAG14	CTCTCGGCGA	162	OPBG02	GGAAAGCCCA
135	OPAG15	CCCACACGCA	163	OPBG03	GTGCCACTTC
136	OPAG16	CCTGCGACAG	164	OPBG04	GTTCCCGACA
137	OPAG17	AGCGGAAGTG	165	OPBG05	CAAGCCGTGA
138	OPAG18	GTGGGCATAC	166	OPBG06	GTGGATCGTC
139	OPAG19	AGCCTCGGTT	167	OPBG07	CAGAGGTTCC
140	OPAG20	GTGAGGCGCA	168	OPBG08	GACCAGAGGT
141	OPAR01	CCATTCCGAG	169	OPBG09	GGCTCTGGGT
142	OPAR02	CACCTGCTGA	170	OPBG10	GGGATAAGGG
143	OPAR03	GTGAGGCGCA	171	OPBG11	ACGGCAATGG
144	OPAR04	CCAGGAGAAG	172	OPBG12	CCCGAGAAAC
145	OPAR05	CATACCTGCC	173	OPBG13	GGTTGGGCCA
146	OPAR06	TGGGGCTCAA	174	OPBG14	GACCAGCCCA
147	OPAR07	TCCTCCGGTG	175	OPBG15	ACGGGAGAGA
148	OPAR08	GTGAATGCGG	176	OPBG16	TGCTTGGGTG
149	OPAR09	GGGGTGTTCT	177	OPBG17	TCCGGGACTC
150	OPAR10	TGGGGCTGTC	178	OPBG18	TGGCGCTGGT
151	OPAR11	GGGAAGACGG	179	OPBG19	GGTCTCGCTC
152	OPAR12	GGATCGTCGG	180	OPBG20	TGGTACCTGG

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

2.5 Clothing and Sequencing of olymorphic brand of somaclonal variants of musa CV grand naine

The polymorphic bands obtained in RAPD markers of dwarf off-type and non-flowering variants were eluted, cloned and sequenced and analysed using Bioinformatics. The transformation of the cloned vector was performed using the GeneJet PCR cloning kit which is compatible with most E. coli laboratory strains.

2.6 Plasmid isolation for sequencing, primer designed and reamplification

Isolation of plasmid from transformed E. coli cells was done using sigma plasmid elution kit. The sequenced polymorphic band data was obtained from MWG biotech lab. Then the sequenced information was matched with NCBI website using blast and fasta tools for DNA and protein homology. Depending on the nucleotide repeats in the sequenced band decamer polymorphic primers were redesigned to 18 nucleotide sequence. Somaclonal variants and true to type banana cv. were amplified using the redesigned primers for confirmation of polymorphism between the variant and true to type banana plants.

3. RESULTS

3.1 Identification of molecular markers specific to somaclones in micropropagated banana CV. Grand naine (MUSA SPP. AAA)

Occurrence of somaclonal variation in micropropagated banana is a serious problem facing the tissue culture industry. In the present study, involves identification of field grown off-types with molecular marker techniques which would help in elimination of such types from micropropagated banana. Experiments were conducted to identify markers specific to the variants using RAPD. Morphological variants like dwarf off-type without bunch, bunch variants were chosen for marker identification as they were causing serious economic loss to the farmers.

3.2 Random amplified polymorphic DNA (RAPD)

DNA samples were collected from different variants along with the normal, true-to-type in-vitro plants checked for purity of DNA samples with Agarose gel electophoresis (Plate 1). Dwarf off-types and normal banana plants were subjected to RAPD in the PCR. 150 primers were screened initially to identify the marker for dwarf off-types since dwarf off-types was the most common somaclonal variant in the farmers' field growing tissue cultured banana plants. Table 2, 3 shows the details of band pattern obtained with 150 primers. 90 primers showed monomorphic bands (Plate 2). Monomorphic bands are those bands which are common to all the samples while polymorphic bands are those that are not common to all. Some of the primers like K4, AR4, AR5 showed monomorphic bands. Usually the number of monomorphic bands ranged from 4-8. Primers OPS5, OPS10, OPS13, OPS16, AR2, AR7, AR14, AG07, BG03, BG9 showed polymorphic bands out of 322 monomorphic bands. No DNA changes were revealed in the RAPD profiles generated with primer OPC1 to 20. Out of 150 primers used 47 primers did not produce any amplification of DNA. OPJ-04 primers were used as reported by earlier work to obtain a polymorphic band in dwarf off-type but no amplification was seen in dwarf off-type and normal plant (Plate 2, Fig. a). Only clear unambiguous and reproducible amplified bands were used for evaluation of the results. The polymorphism of the faint bands present in the RAPD profiles were not considered. Out of the 10 primers which showed polymorphism, OPS 05 primer for dwarf off-types and AR14 primer for dwarf non-flowering type were selected to elute the DNA as the polymorphic bands were intense and reproducible.

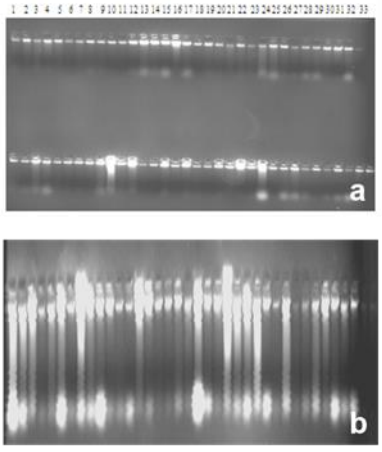


Plate 1.

Figs. a & b Qualitative analysis of DNA samples extracted from different variants and normal plants of banana cv. Grand Naine on Agarose gel electrophoresis.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

a. Genomic DNA samples of true-to-type and various somaclonal variants separated on 1% Agarose gel electrophoresis. Clear bands near the wells indicate good DNA preparation.
b. Purity and integrity of II set of DNA samples tested on Agarose gel electrphoresis, based on molecular weight of extracted genomic DNA samples

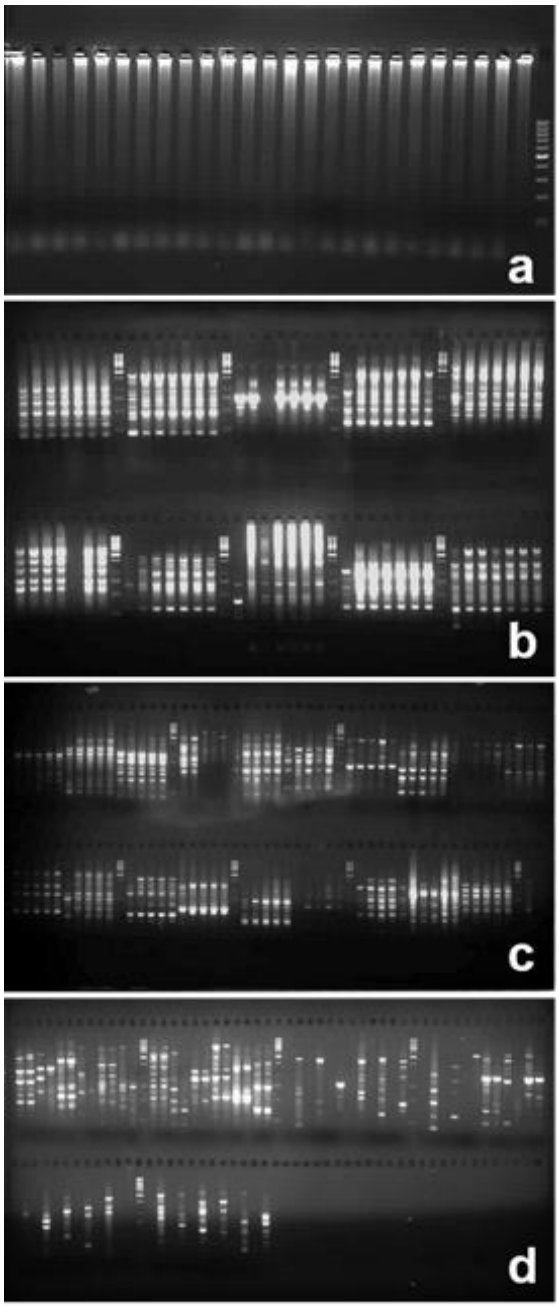


Plate 2.

Figs. a-d: RAPD primers used to generate polymorphic bands in true-to-type and dwarf off-type in vitro propagated banana plants cv. Grand Naine.

a. RAPD primer OPJ-04 used to generate amplification (as reported earlier by Damasco et al., 1996) but the primer OPJ-04 failed to generate bands in both true-to-type as well as dwarf off-types in banana plants.

b. RAPD primers (OPS1-20) used to generate polymorphic bands in true-to-type and dwarf off-type in-vitro propagated banana plants cv. Grand Naine.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

c. 20 RAPD primers used in PCR amplification to generate polymorphic bands between true-to-type and dwarf off-type in-vitro banana plants. Upper lane was loaded with RAPD primers F15, F13, F14, E14, E20, AG7, ARO1, ARO2, ARO3, E12. Lower lane was loaded with OPS1, OPS7, OPS18, OPS 11, OPS 12, A17, D10, A16, AR 20.

d. RAPD primers OPS1-20, K1-K8, E11-E14, F13-F15, E20 were used to amplify DNAs for initial screening purpose to detect polymorphism in dwarf off-types banana plants.

Table. 2. Polymorphic and monomorphic bands amplified by RAPD primers for normal and dwarf off type of banana cv. Grand Naine

S. No.	RAPD Primers	No. of Monomorphic Bands (MB)	No. of Polymorphic Bands (PB)	No. of Poly Morphic Band in True to Types banana	No. of Polymorphic Band in Dwarf off Types banana	No. of MB + PB bands	Total % of Polymorphism
01	OPS 05	3	05	1	4	08	7.4
02	OPS 02	01	02	0	2	03	2.9
03	OPS 07	--	01	1	0	01	1.4
04	OPS 10	4	4	4	0	08	5.9
05	OPS 13	03	03	0	2	06	4.4
06	OPS 16	01	05	0	4	06	7.4
07	AR 7	02	02	0	2	04	2.9
08	AR 2	02	03	1	2	05	4.4
09	AR 14	05	01	0	1	06	1.4
10	K11	03	01	0	1	04	1.4
11	AG 07	04	01	0	1	05	1.4
12	BG 03	05	01	0	1	06	1.4
13	BG 09	03	02	1	1	05	2.9
	Total	36	31	8	21	67	

Table. 3. Monomorphic bands amplified by RAPD primers for normal and dwarf off types of banana cv. Grand Naine

S. No.	RAPD Primer	Monomorphic Bands	S. No.	RAPD Primer	Monomorphic Bands
1	OPS 01	4	28.	AR 12	07
2	OPS 02	3	29	K 19	05
3	OPS 03	4	30.	AR 12	03
4	OPS 04	1	31.	AG 10	04
5	OPS 06	1	32.	K 20	04
6	OPS 07	5	33.	F 15	01
7	OPS 08	3	34.	F 13	03
8	OPS 09	2	35.	E 14	05
9	OPS 11	3	36.	E 20	05

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

10	OPS 12	4	37.	AR 01	01
11	K2	2	38.	AR 02	03
12	K3	3	39.	OPS 01	4
13	K4	2	40.	OPS 07	4
14	OPS 19	2	41.	OPS 18	4
15	AR 6	3	42.	OPS 11	2
16	AG 11	3	43.	OPS 12	2
17	AG 7	1	44.	D 10	05
18	AG 3	4	45.	A 16	02
19	K 14	4	46.	AR 20	01
20	K 13	2	47.	E 12	04
21	K 12	3	48.	BG 1	5
22	OPS 4	1	49.	BG 2	4
23	OPS 8	1	50.	BG 4	04
24	OPS 19	04	51.	BG 5	03
25	OPS 13	5	52.	BG 6	03
26	OPS 15	2	53.	BG 7	01
27	OPS 16	05	54.	BG 10	02
55	BG 11	03	75	BG 18	08
56	BG 12	02	76.	BG 20	05
57	BG 13	03	77.	BG 1	07
58	BG 14	02	78.	BH 2	02
59	BG 15	04	79.	AR 4	02
60	BG 16	03	80.	AR 5	01
61	OPS 14	03	81.	AR 10	02
62	G 02	04	82.	AR 13	02
63	G 06	04	83.	AR 15	02
64	G 05	04	84.	AG 6	04
65	G 07	03	85.	AG 5	03
66	G 09	04	86.	AG 8	04
67	G 16	03	87.	AG 9	2
68	I 16	02	88.	AG 12	03
69	I 17	04	89.	AG 13	02
70	I 20	01	90.	AG 14	01
71	K 17	04	91.	AR 16	01
72	E 15	04			
73	F 14	04			
74	BG 17	04			

Total No. of Monomorphic bands obtained through RAPD primers = 322

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Total No. of Polymorphic bands obtained through RAPD primers = 31
% of Polymorphism observed by using RAPD primers = 9.6

OPS 05 primer having decamer sequence 5' TTT GGG GCCT 3' produced an intense polymorphic band of 750 bp length in all dwarf off-types and was absent in DNA of the normal plant (Plate 3, Fig. a). This polymorphic band was eluted using Sigma elution kit as per manufacturers instruction and sent to sequencing.

The sequence obtained from 750 bp band is given in the Table 4. The sequence data was aligned with NCBI website data. The data base homology search showed that polymorphic sequence obtain in our sequence was in homology with the Musmusculus BAC clone, RP23 from Agrobacterium rhizogene. No protein match was found when aligned in Fasta for protein homology.

Image J software were used to show the amount of difference generated in DNA density graph pattern between true-to-type and dwarf off-type in vitro propagated banana cultivar Grand Naine. Graph created by the primer OPS 05 amplified bands between the normal and variant is shown in the Plate 4, Fig. a & b.

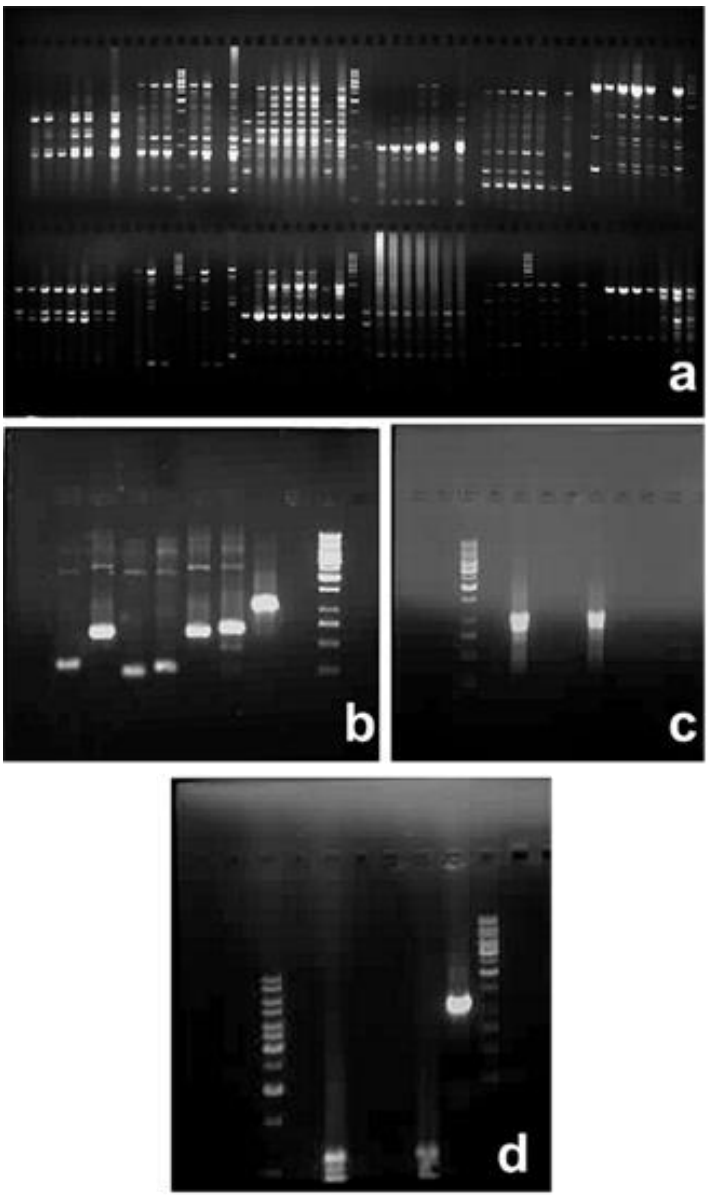


Plate 3.

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

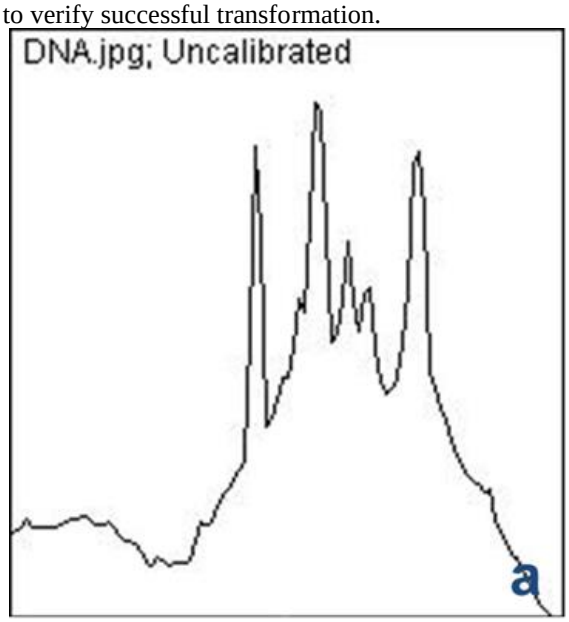
Figs. a-d: Agarose gels showing RAPD primers amplified bands and cloning results of true-to-type and dwarf off-types in-vitro propagated banana plants cv. Grand Naine.

a. RAPD primers OPS 02, OPS 04, OPS 05, OPS 06, OPS 08, OPS 10 were loaded in upper lane sequentially and RAPD primers K2, K3, K4, OPS 19, OPS 20 were loaded in the bottom lane (OPS 05 showed clear 750 kb polymorphic band in dwarf off-type which was absent in true-to-type plant).

b. Gel showing plasmid PCR. Recombinant plasmids having OPS 05 amplified DNA was sent to sequencing and around 5 ul of extracted plasmid was loaded to verify the presence of amplified DNA.

c. Gel showing amplified OPS 05 polymorphic band. Reconfirmation of polymorphic bands (OPS 05 generated) were done with redesigned OPS 05 primer.

d. Gel showing bands generated after colony PCR. PCR conducted after transformation of E. coli cells with pJet vector having OPS 05 amplified DNA to verify successful transformation.



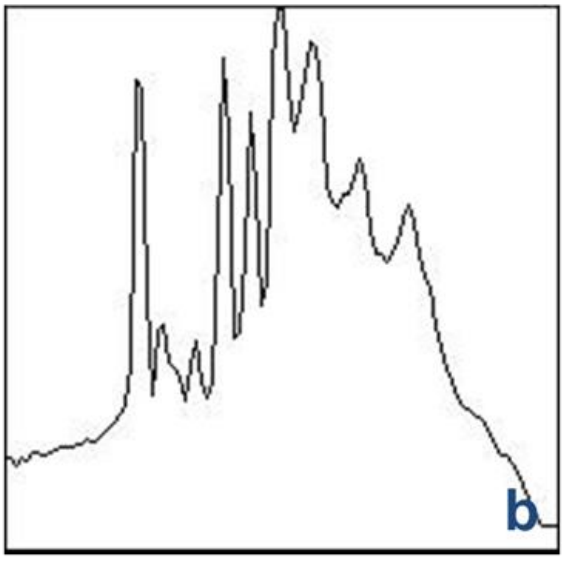


Plate 4.

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Figs. a & b: Graphical representation of density plots of OPS 05 primer generated monomorphic and polymorphic bands in true-to-type and dwarf off-type in vitro propagated banana plants (cv. Grand Naine) using Image J software.
a. Density graph generated in true-to-type plants using amplified bands of OPS 05 primer.
b. Density graph generated in dwarf off-type plants using amplified bands of OPS 05 primer. Variation in the peaks of the graph shows the intensity of variations in DNA amplified between two in vitro plants.

Table. 4. Sequence of polymorphic band amplified through RAPD primer OPS 05

TTGGGGCCTAGAGATGGGGTATTATACTCAGACACCACTAGG
TAACTTGGATGAGTGTCTTGGGGAGATGTATTCAAGACCTAAATACA ACTAGTAGAATAA
ATTAATTAATTAAATTATAAGAAGCTGCATATAGTATTTCCATAGGA GTTAAACTTCCAA
TCGTCTATATTTCTAGAAAAAGTATATATTGCTTTTAGGACAGTTTTC TATAA AAGCTTC
TCCTTATGTGATTTTCCCGATAGGTACTTTTTATTTGGATGGTATCTTT TTTCT AATCCA
TAAATATCCCTCATCATCATCTATCGCTTGCCACTCATCGTTTTTGTC CCTTCGTGGCCA
CCTCCACCTTGCTACTTTCATCGTGCTGCATCTAGGTGGCCACCTTCA CCCCACCATGAG
GCCTCACTGTCTCCTGCCCCAATTGGTAGTGAACTAGGCATGGAGAAT GGCAATATACTAC
TTCTTGGGTCCACCATCGCTCGCTCACTCTCCACTAGTTATGCTCCTCT GTCATGAACT
TAACTGGTTTTGCGTAAGTTGTGCGACACCCTTGCGTGTCCGTCCGT AAAAGGTTAGCCT
CCCCGAAACCTTTTATGGTCCCTTAAAGAACCTACAAAAGAGAAGATA GGTTAGAGAATG
TGTTTAACTCGAGATCCACAAACAGTCATTTCAGCAGACACTTGATAT GCAATATAAAATG
ACAAGCATAGACTTTGAAAGCTCTAAACAGTCGCGCGACAAAGGGT CCTAGGTGGTCCGC
CATGGCCA
GACGGCTOGTOCTOGOGAATGCATCTAGATTTTTGGGCCTATAACAACT ACACCCCATAACATATTAGTA
CTTTTTTGGACGGAGAATAGGCTTAGATGAAGTAGTGGCAGAGCGAAA GCAGAGATACAGTTTACTGTAGG
GTAAGCGAGACTAAAACACCCACAACATAGGAAAGTCAGCCCGAGC TAAGGTGATATCGGCCCTAGTTTTG
AGTTCAAGCTATTAGGGTAGTGTTTCAGTGAAAGCAATACTTGATATAG GGTGCGGGACCCTTAAATGCTA
TTAGGTTTGGTAGCCTTAAAGGCAAGAGTGCCAGTTCGGAGCTACTAAT ATAAGTTCATAGAATAGGGAAT
AGTAGTCGACTAATCACTGACCCGGATCCTTGCTATCAAACAGCCATTC CCGAGCGGAACTACACCTTTCA
CTCTGCATTGACTACTTGCCCAGTTGCTTGGCCGCTGGGCACAGGTAT TGTAATAACAACAATGAAATATA
CTATATTAAGTATATAATGTAAGTTGACTATTGTAGCAAACACAACTTTA GCGCGGCTTGGTGGCTCATGAT
CGAACATAGGCATGCCAAGGGGAAACAAGCTTAGGCATTAATACAAGG AGAGGCTGTGGGTTCCTAACGGG
CAAGACTAAAAGCAAAGATTGTCAGAATCATAGGTCGTAGAGAAAAGG TTCGGGATCCGCATCAGGGGTAGT
ATAAGGACGAGTTGCATTGGGTATGGTCTTAGAAGCAGTGGGTTCAATCT GATAGCAGCGACTCTATTCTT
GTAGCCTCGAGAAAGATGCTAATGC

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Depending on the sequence obtained 10 nucleotides random primer OPS-05 was redesigned to make it 18 nucleotide primer depending on the repetition of the nucleotide in the sequence. The primer OPS-05 was redesigned to 5'-TTT GGG GCC TAG AGA TGG – 3' forward primer, 5'-TTT GGG GCC TAT AAC AAC T'-3' reverse primer (Plate 3, Figs. b-d). The new redesigned primer was obtained from MWG biotech laboratory and reconfirmed using PCR to get RAPD profile for the presence of polymorphic band in the dwarf off-type.

The primer AR-14 having sequence 5'-CTCACAGCAC 3' gave intense polymorphic band of 450 bps in all dwarf non-flowering off-types, which was absent in normal plant (Plate 5, Fig. a). This polymorphic band was eluted and sequenced (Plate 5, Fig. b-d). This sequence obtained was given in Table 5. This sequence was aligned with other DNA sequences in blast of NCBI to find homology with other DNAs. This sequence showed homology with Rhesus macque BAC 250-193 and Astelia menziesiana PsaB gene. The protein homology (protein x or fasta) database showed homology with approprotein A2 of Gossipium barbadense. Twenty primers were used to screen polymorphic bands between malformed bunch variant and normal banana plant. But all of them showed monomorphic bands only.

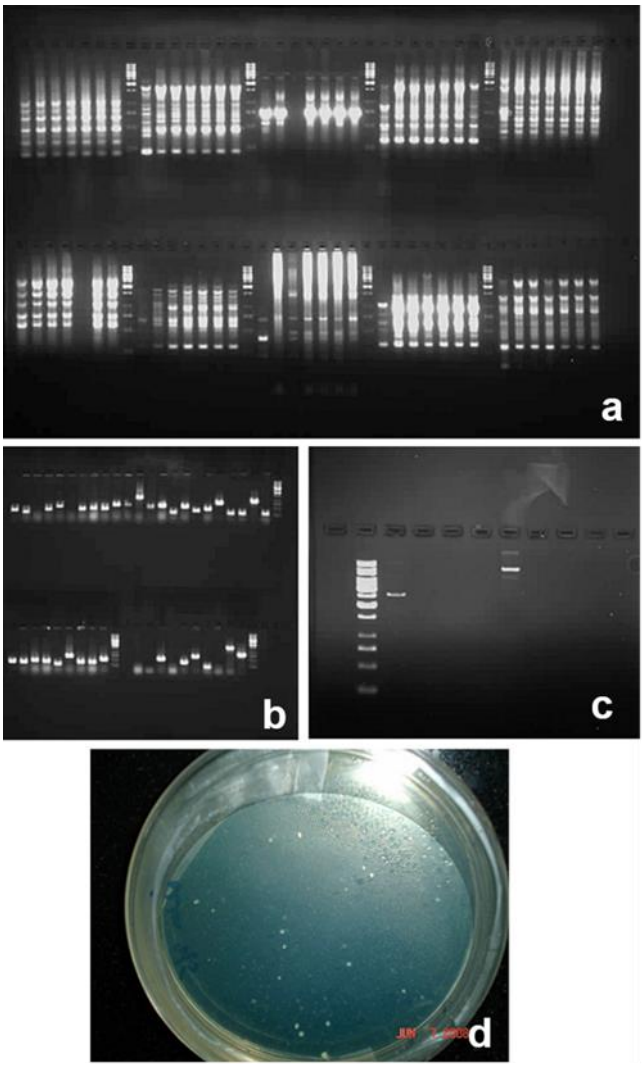


Plate 5.

Figs. a-d: RAPD primers used to identify genetic fidelity between in vitro generated dwarf variant without bunch and normal banana plants of banana cv. Grand Naine.

a. RAPD primers loaded in the upper lane were OPS 19, OPS 13, OPS 15, OPS 17, K 20 and RAPD primers loaded in lower lane were K 19, AR 14, OPS 16, AR 12, AG 10. RAPD primer AR-14 amplified a 450 bp. Polymorphic band in dwarf non-flowering off-type plants and was absent in normal in vitro banana plant. The polymorphic DNA band hence eluted.

b. Gel showing bands formed due to colony PCR. Colony PCR done after transformation to confirm the presence of recombinant pJet vector having AR 14 polymorphic DNA in the bacterial cells.

c. Gel showing plasmid extracted from recombinant bacterial cells.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

d. Petri plate showing white colonies of bacterial cells having recombinant plasmid with AR 14 polymorphic DNA on Luria-bertani medium with ampicillin antibiotic.

Table. 5. Sequence of polymorphic amplified through RAPD primer AR 14

AACATATTAGTACTTTTTTGGACGGAGAATAGGCTTAGATGAAGTAGTG GCAGAGCGAAA
GCAGAGATACAGTTTACTGTAGGGTAAGCGAGACTAAAACACCCACAAC ATAGGAAAGTC
AGCCCGAGCTAAGGTGATATCGGCCCTAGTTTTGAGTTCAAGCTATTAG GGTAGTGTTTC
AGTGAAAGCAATACTTGATATAGGGTGCGGGAGCCTTAAATGCTATTAGG TTTGGTAGCC
TTAAAGGCAAGAGTGCCAGTTCGGAGCTACTAATATAAGTTCATAGAAT AGGGAAT

4. DISCUSSION

4.1 Molecular markers for off-types somaclones in micropropagated banana CV. GRAND NAINE (AAA)

According to INIBAP (2000), the occurrence of somaclonal variation is a serious impediment in the in vitro handling and improvement of Musa. Somaclonal variants are usually inferior and can result in serious economic losses to the farmers through poor yields, compounded by wasted investment of time, field space and other resources in cultivation of propagated plants.[18] The largescale production of commercially elite plants by in vitro micropropagation is technically available for a number of species. A common problem encountered when growing plants by tissue culture is the development of tissue cultured induced genomic polymorphisms in which genetic changes in the nuclear, mitochondria, chloroplast genomes result in the lack of homogenecity among the regenerants and the production of inferior plants that are not true-to-type (off-types) with little commercial value. Many characteristics are only expressed in more mature stage of plant development and not in young stages. Off-types such as dwarfs, thicker leaves are difficult to identify at both tissue culture and nursery stage [13].

Present requirement for confronting somaclonal variation is the early stage markers that could be applied in vitro to identify variation as soon as it occurs. In this direction molecular markers have particularly been suggested to be useful for early stage confirmation of genetic fidelity in micropropagated elite species [19]. The aim of the present study was to provide polymorphic markers for detection of somaclonal variations in tissue cultured banana cv. Grand Naine. Although there are many references dealing with banana tissue culture [2.20,21], the analysis of tissue culture derived plants for somaclonal variations of Indian cvs. are minimum [6,22]. Therefore, study was undertaken to isolate molecular markers for early detection of plants that are not true-to-type. More particularly the work relates to the use of such genetic markers as a diagnostic and quality control tool for monitoring the development of genetic polymorphisms arising during tissue culture regeneration of plants.

As per Gupta and Rajeev, the entire genome cannot be studied on the basis of only one type of marker and each maker system screens only a fraction of the genome and not the whole genome. Hence different markers should be used to screen different fractions of the genome [19]. They state that any failure to detect polymorphism should not be used to infer genetic fidelity but more suitable marker system needs to be assessed in this regard and hence we have used 4 molecular markers to detect the possible polymorphism in the whole genome of micropropagated banana cv. Grand Naine.

The development and use of efficient, reliable methods for detecting off-types is of prime importance to the banana industry. In this regard some of the authors have reported to have isolated molecular markers for off types by RAPD [14], by AFLP [23] by SCAR [24], and by RDA [8] which allows off-types to be discriminated from normal plants [25]. first time used RAPD markers for the assessment of somaclonal variation and reported that variability in banana cvs. having ‘A’ genome was higher than ‘B’ genome. In our study banana cv. Grand Naine (AAA) belongs to ‘A’ genome and it is the most commonly micropropagated plant supplied to the farmers. Hence isolation of markers to detect off-types in banana cv. Grand Naine was taken. Several author have reported somaclonal variations to be genotype specific in banana cvs [26,27], and more particularly to Grand Naine [6].

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 4, April 2022

DOI: 10.17148/IARJSET.2022.9426

Our morphological study of variants in the field revealed dwarf off-type was the most common somaclonal variant in the field, and economically affecting variants like dwarf non-flowering and malformed fingers (small fingers with bitter taste) were also in more numbers and studied for molecular characterization. Many authors have also reported dwarf off-type variant to be the common variant in micropropagated bananas [28,29]. In oil palm non-flowering micropropagated variant has caused enormous loss to the producers [30], along with 10% of regenerants showing mantled flowering in oil palm [31]. Similar non-flowering variant has been recorded in this study and isolation of molecular markers were also tried for such variant along with malformed fingered bunch and bunch variants. Early detection of these variants would help to discard these inferior variants from the nursery fields.

RAPD markers were used to evaluate genetic fidelity of off-types generated by micropropagation of meristems of banana cv. Grand Naine. Random amplified polymorphic DNA techniques has been successfully used for the study of genetic diversity in Indian Musa germplasm [32], and for evaluation of somaclonal variation in Cavendish bananas [6,24]. RAPD technique simply compares the DNA from any number of different samples and can be used to detect the level of difference between them [33].

In the present study initial screening of many variants with true-to-type Grand Naine banana were screened with 150 arbitrary RAPD primers. The result showed many polymorphic bands between the variants rather than normal and the variants, hence screening later was restricted to one variant and true-to-type banana, similar result was also noticed by Gimenez et al. in Williams cultivar [34]. Seven dwarf off-types taken from different fields along with normal pooled true-to-type plant DNA and were subjected RAPD evaluation in this study.

150 primers were used in the initial screening and total of 322 monomorphic bands were formed out of 103 primers. Only 13 primers showed 31 polymorphic bands and the percentage of polymorphism was 9.6%. Newbury et al. reported high levels of RAPD polymorphism (4%) in the tissue culture pedigree with the original cultured meristem of African plantain Agbagba [35]. RAPD technique has been successfully used for assessment of clonal fidelity in maize [36], beet [37], and cotton [38]. Saker et al. reported that in tissue cultured date palm, RAPD analysis showed 4% of genetic variation [39]. The present study 13 primers gave consistency in polymorphic banding pattern, the rest 90 primer formed only monomorphic bands.

Damasco et al. reported that decamer primer OPJ-04 showed polymorphic band of 1.5 kb in all normal plants but was absent in dwarf off-type Cavendish bananas [24]. Similarly, when we tried OPJ-04 primer, no bands were seen both in normal as well as dwarf off type in banana cv. Grand Naine. Bairu et al. reported that OPC-15 shows 1500 kb band consistently in normal plant was absent in all dwarf off-type plants Cavendish banana [40]. No amplification was seen when we used OPC-15 primer to assesses genetic variability in our experiment. Both normal as well as dwarf off-types did not show any bands suggesting the fact that specific primers have to be screened for detection of markers rather than one marker for all the varieties or cvs.

Martin et al. used RAPD finger print to analyse genetic variability of height variants like dwarf, tall, originated from intro cvs. of Grand Naine and petite Naine cvs [41]. He observed polymorphism in dwarf off-type and normal micropropagated plants of Grand Naine and petite Naine cvs. Dwarf off-type Cavendish bananas resulting in the field were identified agronomically as well as by RAPD markers by Gubbuk et al [42]. They used MP12, MP17, MP14 RAPD primers and obtained 49 polymorphic bands out of 80 fragments. In contrast male inflorescence derived plants of Musa accuminata produce no genetic variation with the primers OPC 1 – OPC 20 [43].

In the present study, RAPD primer OPS-05 produced a distinct band of 750 bp in all dwarf off-types from different fields along with 4 other polymorphic bands but were absent in normal banana plant. This distinct band 750 bp was eluted cloned and sequenced. The sequence homology with NCBI database showed similarity with Musmusculus BAC clone RP23 from Agrobacterium rhizogene. No protein match was seen in protein homology database fasta. Similar work was done in rice by Yang et al [44]. The 10 polymorphic bands obtained through RAPD markers between normal and somaclonal variants were cloned and sequenced. Database search showed DNA variations to have occurred in tRNA, ribosomal protein and other genes during cell culture. Distinct variation between DNA density plots of true-to-type and dwarf off-type amplified bands of OPS primer indicate the level of genetic change in in vitro propagated banana cultivar Grand Naine. Further in this study the primer OPS-05 was redesigned to make it 18 mer and tested to determine if they can be used to distinguish individuals from a commercial in vitro propagation facility. A small number of the plants could be distinguished by amplifying a distinct band with the new 18 primer OPS-05. A larger number of individuals need to be tested to confirm the frequency of association between this marker and specific phenotype.

© IARJSET This work is licensed under a Creative Commons Attribution 4.0 International License

DOI: 10.17148/IARJSET.2022.9426

Thomas et al. obtained a dwarf off type markers through representational difference analysis which included substractive hybridization followed by a primer design [8]. The 2 primers could differentiate dwarf off types at the early stage from normal ones. They reported that in Cachaco cultivar dwarf off type amplified a polymorphic band which was absent in true-to-type plants. According to them existence of a particular region in the genome which frequently undergoes number of mutations during in vitro propagation due to the stress. Martin et al. also reported that the somaclonal variant CUDBT-B1 showed distinct band of 1650 bp and was absent in normal Grand Naine banana [6]. The variant CUDBT-B1 showed variegated leaves and early flowering. Another variant CIENBTA-03 William cultivar (AAA) showed tetraploid nature due to in vitro propagation was assessed by RAPD and cytogenetics by Gimenez et al [45].

Present work showed that the bands amplified by the primers OPS 16, OPS 13, OPS 10 showed a distinct polymorphism between dwarf off-type and normal banana plants. Similar polymorphism has been reported by El-doug et al [46]. OPA 2, OPC 5, OPB 4 were used by the above authors to find genetic variations in in vitro propagated Grand Naine cultivar and reported 25% polymorphisms in 8 regenerants. Mohamed S observed that during the 6th sub culture of micropropagated Williams cultivar showed polymorphism as detected by RAPD finger print. The three primers OPK 01, OPK 02, OPK 03 were used by them to detect genetic variability [47]. Ramage et al. (2004) used SCAR marker A1/A21, B1/B21, C1/A2 to detect genetic variability resulted in dwarf off type in banana cv. Williams [48]. According to them genetic variability in off types is because of the tissue culture methodology followed in the laboratories.

Primer AR-14 in this study showed a distinct intense band of 450 bp in all dwarf non-flowering off-types and was absent in normal banana cv. Grand Naine. This band was eluted, cloned and sequenced. Sequence homology with database search showed similarity with apoprotein A2 of cotton. The DNA sequence showed homology with DNA of Rhesus Macque BAC 250-193 and Astelia Menziesiana PsaB gene. Crouch et al. conducted the RAPD assays for the micropropagated banana populations which were generated from single meristem [35]. These were established in field and subjected to intensive RAPD analysis. Based on RAPD analysis classification of individual plants were done and hypothesized that meristems were sectorial chimeras. There was a clear correlation between genotypic variation and tissue culture pedigree. In present investigation RAPD analysis was done for variants like malformed fingers and bunch variants using 30 primers OPC 1-20 and OPD and OPF primers. But distinct polymorphism was not seen with the use of limited primers and also polymorphism was seen between the variants than the normal and the variant, suggesting the scope for use of more different marker system as reported by Rani [49].

5. CONCLUSION

The potential of molecular markers have been used for testing the genetic fidelity of micropropagated cv. Grand Naine (AAA) plants in the fields. Molecular marker like RAPD were used to identify the genetic variations that has resulted in banana cv. Grand Naine. RAPD markers tried clearly showed polymorphism between the off types and the normal banana plants. Overall 9-17% polymorphism was observed in variants indicating the level of genetic change. The primers OPS-05 was redesigned as a marker for dwarf off-type and AR-14 for dwarf non-flowering. However, this is preliminary report on limited number of individuals, and hence a large number of individuals needs to be tested before confirming the association between these markers to a specific genotype.

REFERENCES

[1] Larkin, P. J., & Scowcroft, W. R. (1981). Somaclonal variation—a novel source of variability from cell cultures for plant improvement. Theoretical and applied genetics, 60(4), 197-214.
[2] Smith, M. K., & Hamill, S. D. (1993). Early detection of dwarf off-types from micropropagated Cavendish bananas. Australian Journal of Experimental Agriculture, 33(5), 639-644.
[3] Reuveni, O., & Israeli, Y. (1989). Measures to reduce somaclonal variation in in-vitro propagated bananas. In International Symposium on the Culture of Subtropical and Tropical Fruits and Crops 275, 307-314.
[4] Novak, F. J. (1991). In vitro mutation system for crop improvement. In Plant mutation breeding for crop improvement. V. 2.
[5] Zhengxun, W., & Hongxian, L. (1997). Chromosome aberration in banana micropropagation. Yi Chuan xue bao Acta Genetica Sinica, 24(6), 550-560.
[6] Martin, K.P., Suneesh Kumar, P., Zhang, L.L., Slater, A. and Joseph, M. (2006). RAPD analysis of a variant of banana (Musa sp.) cv. grande naine and its propagation via shoot tip culture. In Vitro Cellular & Developmental Biology-Plant, 42(2), 188-192.
[7] Patil, P. V., & Navale, P. A. (2000). Somaclonal variation in fruit crops: a review. Journal of Maharashtra Agricultural Universities, 25(1), 8-14.

DOI: 10.17148/IARJSET.2022.9426

[8] Thomas, J. Oh, Cullis, C.A., Kunnert, K., Engelborghs, Swenen, R., Cullis, C.A. 2002. DNA markers for the detection of genomic integrity. In 3rd International Symposium on Molecular and Cellular Bio of Banana. Leuven Belgium, Abstracts, 18.
[9] Israeli, Y., Ben-Bassat, and O. Reuveni. (1996). Selection of stable banana clones which do not produce dwarf somaclonal variants during in vitro culture. Scientia Horticulturae, 67(3-4), 197-205.
[10] Uma, S., Selvarajan, R., Saraswathi, M. S., Rameshkumar, A., & Sathiamoorthy, S. (2002). Production of quality planting material in banana. Global Conf. on Banana and Plantain.
[11] Duncan, R. R. (1996). Tissue culture-induced variation and crop improvement. Advances in agronomy, 58, 201-240.
[12] Larkin, P. (2004). Somaclonal variation: origins and causes. Encyclopedia of crop and plant science. Marcel Dekker, New York, 1158-1161.
[13] Vuylsteke, D., Swennen, R., Wilson, G.F. and De Langhe. (1988). Phenotypic variation among in-vitro propagated plantain (Musa sp. cultivar 'AAB'). Scientia Horticulturae, 36(1-2), 79-88.
[14] 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. In International Symposium on Biotechnology of Tropical and Subtropical Species Part 2 461, 157-164.
[15] Porebski, S., Bailey, L.G., Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant molecular biology reporter, 15(1), 8-15.
[16] Mullis, K.B., Faloona, F., Scharf, S.J., Saiki, R.K., Horn, G.T. and Erlkh, H.A. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. In Cold Spring Harbor symposia on quantitative biology, 51, 263-273.
[17] Weblink1: Image J Software http://rsb.inf.nih.gov.ij.
[18] Krikorian, A. D. (1989). In vitro culture of bananas and plantains: background, update and call for information. Trop. Agric.(Trin.), 66, 194-200.
[19] Gupta, P. K., & Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica, 113(3), 163-185.
[20] Banerjee, N., Vuylsteke, D. and De Langhe, E. 1986. Meristem tip culture of musa in : withers LA and Anderson PS (eds.) Butterworths, London, 139-147.
[21] Zaffari, G.R., Pereira Pe RFS, L.F., Fernando, Gilbert, B.K. (2002). Indole-3-acetic acid metabolism in normal and dwarf micropropagated banana plants (Musa spp. AAA). Brazilian Journal of Plant Physiology, 14, 211-217.
[22] 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. Plant cell, tissue and organ culture, 85(1), 11-21.
[23] Engelborghs, I., Swennen, R., & Van Campenhout, S. (1998). The potential of AFLP to detect genetic differences and somaclonal variants in Musa spp. Infomusa, 7: 3-5.
[24] Damasco, P.O., G.C. Graham, R.J. Henry, W., Adkins, K., Smith and I.D. Godwin. (1996). Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (Musa spp. AAA) bananas. Plant Cell Reports, 16(1), 118-123.
[25] Pancholi, N. C., Wetten, A., & Caligari, P. D. S. (1996). Detection of levels of somaclonal variation in Musa using molecular markers. In Meeting on tropical plants, 11-15.
[26] Smith, M. K. (1988). A review of factors influencing the genetic stability of micropropagated bananas. Fruits (France), 219-223.
[27] Vuylsteke, D., Swennen, R., & De Langhe, E. (1991). Somaclonal variation in plantains (Musa spp., AAB group) derived from shoot-tip culture. Fruits, 46(4), 429-439.
[28] Sandoval, J. and Muller, L. 1989. Considerations la conservation in vitro de Musaceae, possibilities and limitations. Association Bananera National (ASBANA) Costarico, 31, 21-24.
[29] Cote, F. X., Sandoval, J. A., Marie, P., & Auboiron, E. (1993). Variations in micropropagated bananas and plantains: literature survey. Fruits, 48(1), 15-23.
[30] Singh, R., Nagappan, J., Rahman, R. A., Tan, S. G., Panandam, J. M., Cheah, S. C., & Board, M. C. (2007). Development of simple sequence of simple sequence repeat markers for oil palm and their application in genetic mapping and fingerprinting of tissue culture clones. In 2007 Conference on Plantation Commodities, Putra World Trade Centre, Kuala Lumpur (Malaysia), Malaysian Cocoa Board, 3-4.
[31] CIRAD. (2003). Centre de co-operation internationale recherché agronomique pour i.e., development. http://www.cirad.fr/en/actualite/communique. php.
[32] Bhat, K. V., Jarret, R. L., & Rana, R. S. (1995). DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. Electrophoresis, 16(1), 1736-1745.
[33] Rekha, A., Ravishankar, K. V., Anand, L., & Hiremath, S. C. (2001). Genetic and genomic diversity in banana (Musa species and cultivars) based on D2 analysis and RAPD markers. Infomusa. WIL O, 2-3.

DOI: 10.17148/IARJSET.2022.9426

[34] Giménez, C., Palacios, G., Colmenares, M., & Kahl, G. (2005). SAMPL: a technique for somaclonal variation fingerprinting in Musa. Plant molecular biology reporter, 23(3), 263-269.
[35] Newbury, H. J., Howell, E. C., Crouch, J. H., & Ford-Lloyd, B. V. (2000). Natural and culture-induced genetic variation in plantains (Musa spp. AAB group). Australian Journal of Botany, 48(4), 493-500.
[36] Osipova, E. S., Koveza, O. V., Troitskij, A. V., Dolgikh, Y. I., Shamina, Z. B., & Gostimskij, S. A. (2003). Analysis of specific RAPD and ISSR fragments in maize (Zea mays L.) somaclones and development of SCAR markers on their basis. Russian Journal of Genetics, 39(12), 1412-1419.
[37] Munthali, M. T., Newbury, H. J., & Ford-Lloyd, B. V. (1996). The detection of somaclonal variants of beet using RAPD. Plant cell reports, 15(7), 474-478.
[38] Jin, S., Mushke, R., Zhu, H., Tu, L., Lin, Z., Zhang, Y., & Zhang, X. (2008). Detection of somaclonal variation of cotton (Gossypium hirsutum) using cytogenetics, flow cytometry and molecular markers. Plant Cell Reports, 27(8), 1303-1316.
[39] Saker, M. M., Bekheet, S. A., Taha, H. S., Fahmy, A. S., & Moursy, H. A. (2000). Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biologia Plantarum, 43(3), 347-351.
[40] Bairu, M. W., Fennell, C. W., & van Staden, J. (2006). The effect of plant growth regulators on somaclonal variation in Cavendish banana (Musa AAA cv.'Zelig'). Scientia Horticulturae, 108(4), 347-351.
[41] Grajal-Martin, M., Siverio-Grillo, G., & Marrero-Domínguez, A. (1997). The use of randomly amplified polymorphic DNA (RAPD) for the study of genetic diversity and somaclonal variation in Musa. In II International Symposium on Banana: I International Symposium on Banana in the Subtropics 490, 445-454.
[42] Gubbuk, H., Pekmezci, M. U. S. T. A. F. A., Onus, A. N., & Erkan, M. U. S. T. A. F. A. (2004). Identification and selection of superior banana phenotypes in the cultivar Dwarf Cavendish using agronomic characteristics and RAPD markers. Pakistan Journal of Botany, 36(2), 331-342.
[43] Harirah, A. A., & Khalid, N. (2006). Direct regeneration and RAPD assessment of male inflorescence derived plants of Musa acuminata cv. Berangan. Asia Pacific Journal of Molecular Biology & Biotechnology, 14(1), 11-17.
[44] Yang, H., Tabei, Y., Kamada, H., Kayano, T., & Takaiwa, F. (1999). Detection of somaclonal variation in cultured rice cells using digoxigenin-based random amplified polymorphic DNA. Plant Cell Reports, 18(6), 520-526.
[45] Giménez, C., De Garcia, E., De Enrech, N. X., & Blanca, I. (2001). Somaclonal variation in banana: cytogenetic and molecular characterization of the somaclonal variant CIEN BTA-03. In Vitro Cellular & Developmental Biology-Plant, 37(2), 217-222.
[46] El-Dougdoug, K. A., El-Harthi, H. M. S., Korkar, H. M., & Taha, R. M. (2007). Detection of somaclonal variations in banana tissue culture using isozyme and DNA fingerprint analysis. Journal of Applied Sciences Research, 3(7), 622-627.
[47] Mohamed, A. E. (2007). Morphological and molecular characterization of some banana micro-propagated variants. International Journal of Agriculture and Biology (Pakistan). Int. J. of Agr. Biol., 707-714.
[48] 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 (Musa spp. AAA). Scientia Horticulturae, 103(1), 145-151
[49] Rani, V., & Raina, S. N. (2000). Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. In Vitro Cellular & Developmental Biology-Plant, 36(5), 319-330.