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.2022.9427

Detection of Somaclonal Variations in Banana CV. Grand Naine (AAA) Using Simple Sequence Repeats (SSR) Molecular Marker 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: The main purpose of present study was detection of variations of somaclones in the banana cv. Grand Naine by using simple sequence repeats (SSR) 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 SSR 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 SSR, markers are used to assess the genetic variation in somaclonal variants of banana cv. Grand Naine. Out of 78 SSR primers screened to detect polymorphism, 11 primers showed polymorphism and 45 SSR primers showed only monomorphic bands. In conclusion, the potential of molecular markers has been used for testing the genetic fidelity of micropropagated cv. Grand Naine (AAA) plants in the fields. Molecular marker like SSR were used to identify the genetic variations that has resulted in banana cv. Grand Naine. SSR markers tried clearly showed polymorphism between the off types and the normal banana plants. In conclusion, overall 17% polymorphism was observed in variants indicating the level of genetic change.

Keyword: Molecular markers, Polymorphism, SSR, Grand naine (AAA)

1. INTRODUCTION

Banana is most widely grown fruit crop in tropical and subtropical regions [1], and micro-propagated banana planting material offers several advantages over vegetative propagation. Somaclonal variations, either genetic or epigenetic in nature, have been considered an integral part of in-vitro regeneration process. Somaclonal variants are an important source of genetic variation especially in vegetatively propagated plants and have been used as a tool for plant improvement as well as development of new varieties [2-4]. Important features for which in-vitro propagation are opted is its enormous multiplicative capacity in a relatively short span of time, production of healthy and disease free plants and its ability to generate propagules throughout the year [5]. These variations are often undesirable in a tissue culture industry where the main aim is production of "true to type" plants. Characterization of off-type variants for traits superior to mother plants in terms of yield, fruit quality, resistance to biotic or abiotic stresses and higher regeneration efficiency in tissue culture media might lead to development of new cultivar. Dwarf Cavendish, Grand Naine Israel, Lancefield, Chinese Cavendish are some of the proven examples where somaclonal variants/mutants have been characterized, selected and released as new variety in different parts of the world.

However, some of the somaclonal variants also show characteristic feature inferior to the mother plants and causes heavy yield and economic loss to farmers as well as tissue culture industry. It is therefore important to characterize, select and assess the somaclonal variants for presence of elite and off types traits observed during in-vitro regeneration or in farmers' field. Morphological description, physiological supervision, karyotyping, biochemical estimations and field assessment have been used conventionally to describe and characterize such somaclonal variants. Since most of these traits are governed by many genes having additive or environment interactive effects, they remain highly subjective and difficult to identify. Recently molecular characterization and identification techniques are being used for efficient and effective management of plant genetic resources. Genetic uniformity of in-vitro raised plants also a prerequisite for production of quality planting material of banana [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.2022.9427

Somaclonal variation arising from in-vitro production of plantlets is associated with DNA novel and heritable phenotypic variation which could be epigenetic or genetic [7]. The present day molecular markers have complemented traditional methods to detect genetic variants, monitor genetic fidelity and varietal development of asexually propagated plants. Thus, DNA markers based characterization of somaclonal variants showing discrete elite or off-type characters followed by identification of markers associated to superior traits (if any) would be extremely beneficial. With this scenario, the present study was undertaken with main aim of detection of variations of somaclones in the banana cv. Grand Naine by using simple sequence repeats (SSR) molecular marker technique.

2. MATERIALS AND METHODS

2.1 Source of Planting Materials

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 (Cetyl Trimethyl 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 [8].

2.3 PCR Amplification of DNA

Polymerase chain reaction (PCR) 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 94-98°C, b) Annealing of primers to template DNA at 35-55°C and c) Primer extension from their 3' ends at 72°C. 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 [9].

2.4 SSR to Detect Genetic Variation in Micropropagated Banana cv. Grand Naine

SSR are highly variable and evenly distributed throughout the genome. This type of repeated DNA is common in Eukaryotes, their number of repeated units varying widely among organisms as high as 50 copies of the repeated units. Fragments containing microsatellites can be amplified by the PCR using a pair of primers flanking the repeat sequence. The polymorphism between genotypes is due to the variation in the number of repeat units. SSR are one the markers used to characterize the genetic variability of the genus Musa, because they are highly polymorphic, multiallelic, co-dominant, reproducible, easy to interpret and amplified via PCR [10]. In the present study 78 SSR primers have been used to record the genetic variability in somaclones of micropropagated banana cv. Grand Naine. The list and sequence of the primers used are given in the Table 2.

Table. 2. SSR Primer Sequences Used in Screening

1F	5' – GGT TGG AAC GGA ATA CTA A – 3'
1R	5' – TCC AAG CTT ATC GAT CTA CG – 3'
2F	5' – TGT CGA AGC ATC CTA CAT C – 3'
2R	5' – CTT GGA AAC ATG AGA AAC ATA C – 3'
3F	5' – TTG AAG TGA ATC CCA AGT TTG - – 3'
3R	5' – AAA ACA CAT GTC CCC ATC TC - – 3'
4F	5' – TGC TCT TCC ACA TCT CAA GAA C – 3'
4R	5' – GAT TGC ACG GAG ATT CAA CA – 3'
5F	5' – GGT GCT CTT CGG AGG A – 3'
5R	5' – CGC TTT ATA TCC ATT CCC A – 3'
6F	5' – CGG AAG TGG CG GGT AGA GA – 3'
6R	5' – CCC AAC AAC TTA TGG CGG AGA – 3'
7F	5' – CCT CTT CTC TCC TTC ACT TTC TCA – 3'

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.2022.9427

7R	5’ – AGG ATG GCG GAG ATC TGG TCA – 3’
8F	5’ – CTA CAA CAA TAA TCC AGG GCA A – 3’
8R	5’ – GGT CAT CAC GGC GTT CTC CA – 3’
9F	5’ – GTG TGT GTG TGT GTG TGT GTG A – 3’
9R	5’ – GAA TTC GTG CTT TCT GTT TGG TGA – 3’
10F	5’ – GTC GAA CTC CTT CCA CTT CCA – 3’
10R	5’ – TAT GTA GGC TGT GTG TGT GTC TGA – 3’
11F	5’ – CCA GCG ATA CCC TTC ATG ACC A – 3’
11R	5’ – CTG ATT AGG ATT TGA AAG GGG CAA – 3’
12F	5’ – GTT CAC ATG AAG ACC GGG CAA – 3’
12R	5’ – CTC TCG ATG GGT TTC CCA AGG A – 3’
13F	5’ – AAT GGC TGC CTG CCA TGA A – 3’
13R	5’ – GAA TCA AAG AGG CGA GAA GAC GA – 3’
14F	5’ – CTG CCT CTC CTT CTC CTT GGA A – 3’
14R	5’ – TCG GTG ATG GCT CTG ACT CA – 3’
15F	5’ – TCG GCT GGC TAA TAG AGG AA – 3’
15R	5’ – TCT CGA GGG ATG GTG AAA GA – 3’
16F	5’ – AGG TGC CAC ACA GTT CAG ACA – 3’
16R	5’ – CAA CCC AAA CCT GTT CGA CCA A – 3’
17F	5’ – ACT TGG GAC GTC GAA GCA – 3’
17R	5’ – TGT GCT GGA ATT CCC TGA – 3’
18F	5’ – CAC GGT TTT TCT TGC CCC GAA – 3’
18R	5’ – TTC CAC GTG CAA GCC TGC A – 3’
19F	5’ – ATG CCC AAG AAG GGA AGG GAA – 3’
19R	5’ – TAA TGC CGG AGG ATC AGT GTG A – 3’
20F	5’ – AGG GAG GGA TCA GAA GGA GCA – 3’
20R	5’ – CGG CTT CCT CTG AAG GTT CCA – 3’
21F	5’ – CCG TTG GAT TTC TCC CCC ACA – 3’
21R	5’ – ATT CGA GGC ATC GTC GTC CA – 3’
22F	5’ – GAA GGT TCC CAG AAT CGA TAG TGA – 3’
22R	5’ – AGA TTC CGT TTC CGT TGC TA – 3’
23F	5’ – AGT TTA TTC GGT GGA CGT TAA CGA – 3’
23R	5’ – GAT CTG TGT ATT TGG TCG ACG GTA – 3’
24F	5’ – TTC GTG ACG CAA GCC TGA – 3’
24R	5’ – CCG AAA CGA AGG TTA CAA CAA – 3’
25F	5’ – GCG CCA CCT GTA TCA CTG T – 3’
25R	5’ – AGC AGA CCC ATC GAG ATA CG – 3’
26F	5’ – TTC TCT TTG CCT CGT TGC TT – 3’
26R	5’ – AGT GAG TGT CCC CAA ACG TGA – 3’

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.2022.9427

27F	5’ – TAA AAT GTG CAA ATG GGC GTG GA – 3’
27R	5’ – TCA CAT ACC GAA CAG AGA GAG TCA – 3’
28F	5’ – CCG ACC GTG AAC TTC TTT TCC A – 3’
28R	5’ – ACT AAC TGA GGA GAA TCA AGG AGA – 3’
29F	5’ – AAA CCG CCT CTA CAT CCG GAA – 3’
29R	5’ – GCG GTC AAG GAT CCG ACC AA - 3’
30F	5’ – GGC TTC CTC TGA AGG TTC CAG A – 3’
30R	5’ – GAA GAA CTG GGC TTA CCC AGG A – 3’
31F	5’ – GAT GAT GGT GAG AGG CTG ATG A – 3’
31R	5’ – GGT CGG TAT GGG AAG CAC C – 3’
32F	5’ – TTT GCC TGG TTG GGC TGA – 3’
32R	5’ – CCC CCC TTT CCT CTT TTG C – 3’
33F	5’ – AGG CGG GGA ATC GGT AGA – 3’
33R	5’ – GGC GGG AGA CAG ATG GAC T – 3’
34F	5’ – TGA ATC CCA AGT TTG GTC AAG – 3’
34R	5’ – CAA AAC ACT GTC CCC ATC TC – 3’
35F	5’ – CTC CTT TGT GAG CTC GGC ATA T – 3’
35R	5’ – AGG GTC CAA GAA ACT CCT CCA A - 3’
36F	5’ – TGA ATC CCA AGT TTG GTC AAG A – 3’
36R	5’ – CCA ACT CTT GTC CCT CAC TTC A – 3’
37F	5’ – GAA GCA TCC AAT GGA CCT A – 3’
37R	5’ – GCG AAC TCA CAA TAG CGA – 3’
38F	5’ – CTT TGG AAG GTG GTT CTC A – 3’
38R	5’ – ACG ACT GAG ACC GAT TGA G – 3’
39F	5’ – CCC GTC CCA TTT CTC A – 3’
39R	5’ – TTC GTT GTT CAT GGA ATC A – 3’
40F	5’ – GGT GGA TGG CTG GGT A – 3’
40R	5’ – GGA TCC AAG CTT ATC CGA GTT – 3’
41F	5’ – GGT GCT CTT CGG AGG A – 3’
41R	5’ – CGT TTA TAT CCA TTC CCA – 3’
42F	5’ – GCT TGT CTC TCA CCC ACT C – 3’
42R	5’ – ACC GAC TCC CCA ATA GG – 3’
43F	5’ – TGG CTG ACA ATT ACA TGA CA – 3’
43R	5’ – GCG CAC TGT GGT GTG T – 3’
44F	5’ – AAA TCG AAA AGT GGA TAA AAA CTA A – 3’
44R	5’ – CCT CAC ACC AAC ACA A – 3’
45F	5’ – CTG GTC CTT TTC AGT TCA CTC – 3’
45R	5’ – TAG GCA AGC TCC CAA TCA – 3’
46F	5’ – CTA GGC TTC CTG CTG CTC – 3’

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.2022.9427

46R	5’ – TGA GCG AAT TTG ATC AGA AC – 3’
47F	5’ – GCA CGA AGA GGC ATC AC – 3’
47R	5’ – GGC CAA ATT TGA TGG ACT - 3’
48F	5’ – CAC GCG GTT CCT TCT C – 3’
48R	5’ – TCC GGA TCC AAG CTT ATC – 3’
49F	5’ – TCG CCT CTC TTT AGC TCT G – 3’
49R	5’ – TGT TGG AG ATC TGA GAT TG – 3’
50F	5’ – AGA ACG TTT GCT GTT GGA G - 3’
50R	5’ – GCT TCT GTC ATC GTT TTG TC – 3’
51F	5’ – ATT TCC TGC TGA TTA TGA CTC TT – 3’
51R	5’ – TCG CTCTAA TCG GAT TAT CTC – 3’
52F	5’ – TCT CCG GAT CCA AGC TTA – 3’
52R	5’ – TCT CCG GAT CCA AGC TTA – 3’
53F	5’ – ACT GCT GCT CTC CAC CTC AAC – 3’
53R	5’ – GTC CCC CCA GAA CCA TAT GAT T - 3’
54F	5’ – ACG GTG ATG AAA GCT TAC ACG – 3’
54R	5’ – GTG GCC GAA AAC ACA ACA ACC – 3’
55F	5’ – ATT GGG CAG GCA TCA AGT AC – 3’
55R	5’ – ATT GGG CAG GCA TCA AGT AC – 3’
55R	5’ – GCA ATG GTG CTA CCC ACC – 3’
55R	5’ – GCA ATG GTG CTA CCC ACC – 3’
56F	5’ – AGT CAC GGA GCA TAT TTG GG – 3’
56R	5’ – TAC TCA AGC TAT GCA GCA TCC AAC G – 3’
57F	5’ – TCA CGA AAC ACT GAA AAG CG – 3’
57R	5’ – TTT TCC TCC CCG GAA AAG – 3’
58F	5’ – GGG TTC CGT GAA GAT TGA TT – 3’
58R	5’ – TGG ACA ACT GAC CAT AAT – 3’
59F	5’ – ACG ATC TGG CTG AGA ATT GG – 3’
59R	5’ – TCT CTA TGG ATT GAA ACC ACC C – 3’
61R	5’ – TCC CTC TTC AAC CAA AGC AC – 3’
62F	5’ – AAA CGT GAA ACG ACA GCT TCT G – 3’
62R	5’ – TCC GGC TTC GAA TTG AAG G – 3’
63F	5’ – AAC AAC TAG GAT GGT AAT GTG TGG AA – 3’
63R	5’ – GAT CTG AGG ATG GTT CTG TTG GAG TG – 3’
64F	5’ – TGC AGT TGA CAA ACC CCA CAC A – 3’
64R	5’ – TTG GGA AGG AAA ATA AGA AGA TAG A – 3’
65F	5’ – ACA GAA TCG CTA ACC CTA ATC CTC A – 3’
65R	5’ – CCC TTT GCG TGC CCC TAA – 3’
66F	5’ – AAT CGA AAT CGA GTC AAC AAG G – 3’

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.2022.9427

66R	5’ – TTT TGT GGA TGG TTG GTT CC – 3’
67R	5’ – TTA AAG GTG GGT TAG CAT TAG G – 3’
68F	5’ – TGA CCC ACG AGA AAA AGA AGC – 3’
68R	5’ – CTC CTC CAT AGC CTG ACT GC – 3’
69F	5’ – ACT TAT TCC CCC GCA ACT CAA – 3’
69R	5’ – ACT CTC GCC CAT CTT CAT CC – 3’
70F	5’ – AGT TTC ACC GAT TGG TTC AT – 3’
70R	5’ – TAA CAA GGA CTA ATC ATG GGT – 3’
71F	5’ – TCC CAA CCC CTG CAA CCA CT – 3’
71R	5’ – ATG ACC TGT CGA ACA TCC TTT – 3’
72F	5’ – TC CAT AAG TGT AAT CCT CAG TT – 3’
72R	5’ – CTC CAT CCC CCA AGT CAT AAA G – 3’
73F	5’ – AAG TTA GGT CAA GAT AGT GGG ATT T – 3’
73R	5’ – CTT TTG CAC CAG TTG TTA GGG – 3’
74F	5’ – GGA GGC CCA ACA TAG GAA GAG GAA T – 3’
74R	5’ – CAT AAA CGA CAG TAG AAA TAG CAA C – 3’
75F	5’ – TGA GGC GGG GAA TCG GTA – 3’
75R	5’ – GGC GGG AGA CAG ATG GAG TT – 3’
76R	5’ – AGC CAT ATA CCG AGC ACT TG – 3’
77F	5’ – ATG TCG CTT CGG ACC AGA - 3’
77R	5’ – GCA GGA CGA AGA ACT TAC C – 3’
78F	5’ – ATG ATC ATG AGA GGA ATA TCT – 3’

3. RESULTS

3.1 Identification of Molecular Markers Specific to Somaclones in Micropropagated Banana cv. Grand Naine

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 SSR. 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 Simple Sequence Repeats (SSR)

To determine the genetic variation in the dwarf off-type micropropagated banana, 78 SSR primers were employed (Table 1 and 2). shows the number of monomorphic and polymorphic bands obtained with these primers. Almost all the primers showed 1 or 2 monomorphic bands. Out of 78 SSR primers screened to detect polymorphism, 11 primers showed polymorphism and 45 SSR primers showed only monomorphic bands (Plate 1, Fig. a). 22 SSR primers did not show any amplification. SSR primers 12, 5, 28, 53, 56, 66 showed polymorphic bands (Plate 2, Fig. c & d). Polyacrylamide gel was used to separate the SSR amplified bands since these bands were lesser than 500 bps and PAGE resolves the smaller molecular weight DNA more effectively. Intense polymorphic bands were seen in dwarf off-types with primers 8, 33, 35, 46, 68 (Plate 2, Fig. a) but primers 31, 75, 26, 29 showed monomorphic bands. The number of monomorphic bands separated or resolved on page was 1-8 compare to agarose gel where the number of monomorphic bands separated were one or two. Monomorphic bands were seen when primers (47, 48, 49, 50, 51, 52, 53, 34, 75, 76, 74, 73, 24, 77) were used to screen non-flowering off-type and normal banana plant SSR primers 6, 58, 60, 70, 73, 79, 63 showed monomorphic bands only when screened to detect variation between malformed bunch and normal banana plant (Plate 2, Fig. b).

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.2022.9427

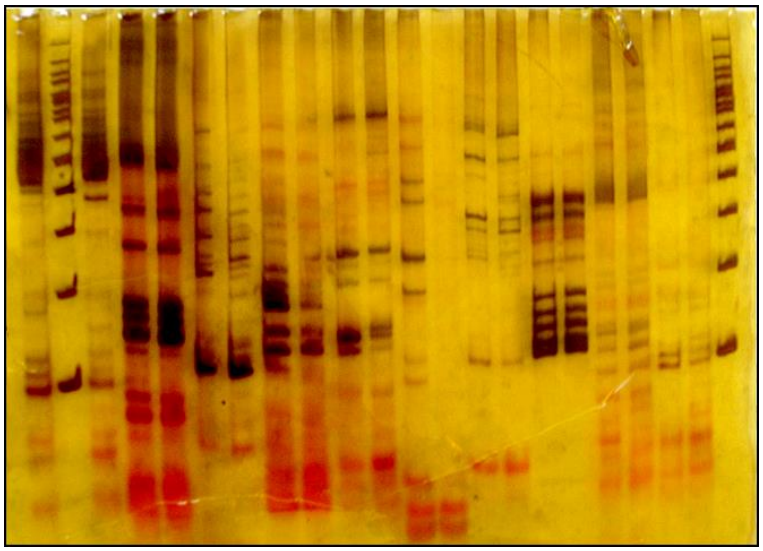

Plate-1: PAGE showing amplification of DNA by SSR primers in true-to-type and dwarf off-types in vitro propagated banana cv. Grand Naine.
PAGE (polyacrylamide gel electrophoresis) showing DNA amplification by SSR primers 8, 31, 33, 35, 46, 66, 68, 75, 26, 29 in true-to-type and dwarf off-type banana.

SSR primers 8, 33, 35, 46, 66, 68 showing polymorphism.

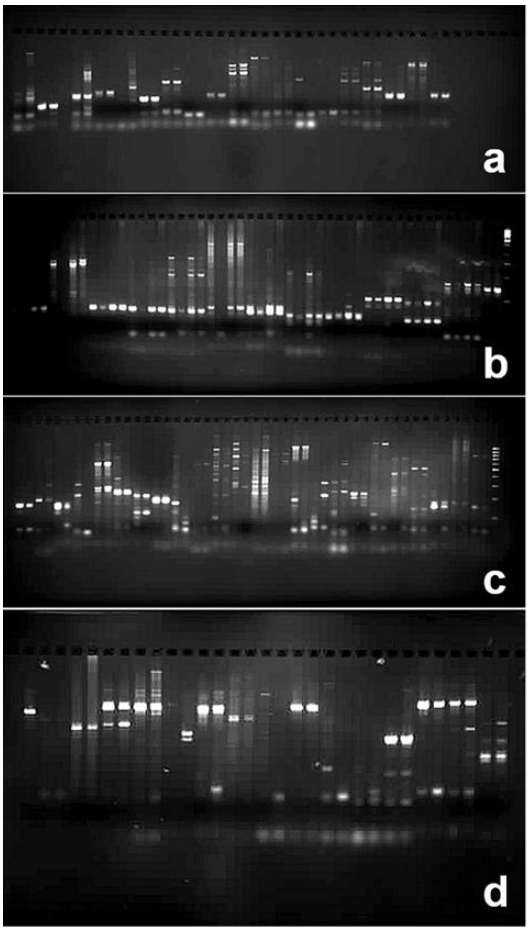

Plate 2

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.2022.9427

Figs. a-d SSR primers amplified polymorphic and monomorphic bands in true-to-type and dwarf off-type in vitro propagated banana plants cv. Grand Naine.

a. Gel showing amplification done by SSR primers. SSR primers 31 to 49 series used to amplify polymorphic bands between normal and dwarf off-type.

b. Gel showing amplification of DNA using SSR primers 47 to 54, 75-77.

c. Agarose gel showing amplification of DNA using SSR primers 61-69, 51-57, 02, 20, 15, 43, 29, 26, 31, 33, 35, 46, 74.

d. Gel showing amplification of DNA by SSR primers of true-to-type and dwarf off-type banana plants. SSR primers 8, 10, 9, 11, 12, 15, 16, 18, 19, 20, 22, 25, 27, 28, 75 were used.

Table. 1. Polymorphic and monomorphic bands amplified by SSR primers for normal and dwarf off type by banana cv. Grand Naine

S. No.	SSR Primers	MB	PB	PB in true to types banana	PB in dwarf off types banana	MB + PB bands	Polymorphism (%)
1	5	1	1	1	0	2	1.47
2	8	8	3	1	2	11	4.40
3	12	1	1	0	1	2	1.47
4	28	1	1	0	1	2	1.47
5	29	5	1	0	1	6	1.47
6	35	8	2	2	0	10	2.90
7	46	6	3	1	2	9	4.40
8	53	1	1	1	0	2	1.47
9	56	1	2	1	1	3	2.90
10	66	2	2	2	0	4	2.90
11	68	5	2	2	0	7	2.90
12	33	8	2	0	2	10	2.90
	Total	47	21	11	10	68	

MB: Mono-morphic bands; PB: Poly-morphic bands

Table. 2. Monomorphic bands amplified by SSR primers for normal and dwarf off-types of banana cv. Grand Naine

S. No.	SSR Primer	No. of MB	S. No.	SSR Primer	No. of MB
1.	3	1	26.	41	1
2.	4	1	27.	45	3
3.	6	1	28.	47	1
4.	7	2	29.	48	1
5.	9	1	30.	49	1
6.	11	2	31.	54	2
7.	14	1	32.	55	1
8.	15	2	33.	58	1
9.	16	1	34.	60	1
10.	17	1	35.	61	1

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.2022.9427

11.	19	1	36.	62	1
12.	23	1	37.	63	1
13.	24	1	38.	64	1
14.	25	1	39.	65	1
15.	26	1	40.	67	1
16.	27	2	41.	69	1
17.	30	1	42.	70	1
18.	31	8	43.	71	1
19.	32	1	44.	72	1
20.	34	1	45.	73	2
21.	36	1	46.	74	2
22.	37	2	47.	75	9
23.	38	1	48.	77	1
24.	39	1	49.	78	1
25.	40	3			

MB: Mono-morphic bands; PB: Poly-morphic bands;

Total No. of Monomorphic bands obtained through SSR primers = 123
Total No. of Polymorphic bands obtained through SSR primers = 21
% of Polymorphism observed by using SSR primers = 17

DISCUSSION

According to Daniells, 2001 [11], 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 [12]. 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 [14]. 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 [15-17], the analysis of tissue culture derived plants for somaclonal variations of Indian cvs. are minimum [18,19]. 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.

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 [20,21]. In oil palm non-flowering micropropagated variant has caused enormous loss to the producers [22], along with 10% of regenerants showing mantled flowering in oil palm [23]. Similar non-flowering variant has been recorded in this study and isolation of

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.2022.9427

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.

Simple Sequence Repeat (SSR) markers have been described as reliable, reproducible, cost effective co-dominant, locus specific and have highest discriminative ability compared to other marker systems. The importance of SSR markers to assess the genetic fidelity of tissue culture derived plants has been reported for other crops such as populus [24], and Pinus [25]. Microsatellites are among several molecular markers used to characterize the genetic variability of the genus Musa because they are highly polymorphic [26]. In recent years a variety of molecular markers based on microsatellites have become the markers of choice. We have used 78 SSR primers to detect genetic diversity in dwarf off-type micropropagated banana cv. Grand Naine. Total of 123 monomorphic bands were given by 45 SSR primers and 21 polymorphic bands were given by 11 SSR primers. Therefore, the percentage of polymorphism recorded was 17%.

Singh et al. used SSR markers in DNA finger printing of tissue cultured clones of oil palm for detecting culture mix-up, monitor line uniformity and to detect clonal fidelity of oil palm [27]. They reported that SSR can distinguish tissue cultured ramets and ortes cvs. Palombi and Damiano reported that SSR alone could detect variation induced in micropropagated kiwi fruit, compare to RAPD [28]. The effective detection of somaclonal variation in Populus tremuloides was shown by Rahman and Rajora [24]. According to them even though micropropagated populus did not show morphological variation, SSR finger print detected duplication of a chromosomal segment in the tissue culture plants. Hence they state that somaclonal variations cannot always be detected at the gross morphological level but with sensitive makers like SSR can determine the clonal fidelity.

In our study PAGE was used to separate the amplified SSR bands as resolution of DNA bands on acrylamide gels was superior to that of agarose gel and silver staining was used as it is more sensitive than ethidium bromide. Hollingsworth et al. used acrylamide gel to detect somaclonal variation in Asparagus [29]. In the present study primers 8, 28, 29, 35, 46, 66, 68 showed more than 6 bands when resolved on acrylamide gels. Detection and quantification of in vitro induced chimerism of Theobroma cacao was done by Rodriguez Lopez et al. using SSR marker and reported 31% of chimeric mutants due to the loss of allele or slippage mutation [30]. Gupta and Varsheny 2000 reported to have used 23 SSR primers to detect polymorphism in wheat somaclonal variants and observed the reproducible polymorphism in them with the use of SSR markers [14]. In this study monomorphic bands were seen with SSR primers 6, 58, 59, 63, 70, 73, 79 in case of malformed finger variant. Similarly, primers 47-54, 75-77 showed monomorphic bands in dwarf non-flower variant. Genetic uniformity Eucalyptus micropropagated plants was detected by SSR markers [31]. Regenerated cotton plants through somatic embryogenesis showed high somaclonal variation which was detected by SSR and RAPD markers [32]. As such, the application of the SSR primer for the use as quality control tool can be evaluated by the above reports.

CONCLUSION

The potential of molecular markers has been used for testing the genetic fidelity of micropropagated cv. Grand Naine (AAA) plants in the fields. Molecular marker like SSR were used to identify the genetic variations that has resulted in banana cv. Grand Naine. SSR markers tried clearly showed polymorphism between the off types and the normal banana plants. Out of 78 SSR primers screened to detect polymorphism, 11 primers showed polymorphism and 45 SSR primers showed only monomorphic bands. Overall 17% polymorphism was observed in variants indicating the level of genetic change. However, this is a 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] Kalloo, G. "Banana and plantation research in India–a perspective. Global Conf. on Banana and Plantain." (2002), 28-31.
[2] Cullis, C. A. "The molecular biology of plant cells and cultures." Plant biotechnology. Comprehensive biotechnology, second supplement. (1992), 19-32.
[3] Karp, A. "Are your plants normal? Genetic instability in regenerated and transgenic plants." Agro-Food-Industry Hi-Tech May/June (1993), 7-12.
[4] Cassells, A. C., Joyce, S. M., Curry, R. F., & McCarthy, T. F. "Detection of economically important variability in micropropagation." Plant Biotechnology and in vitro Biology in the 21st Century. Springer, Dordrecht, (1999), 241-244.
[5] Dhawan, V. I. B. H. A., and SANT S. Bhojwani. "Micropropagation in crop plants." Glimpses Plant Res 7 (1986), 1-75.

DOI: 10.17148/IARJSET.2022.9427

[6] Rout, G. R., Senapati, S. K., Aparajita, S., & Palai, S. K. "Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers." Plant Omics 2.6 (2009), 250-285.
[7] Lukens, Lewis N., and Shuhua Zhan. "The plant genome's methylation status and response to stress: implications for plant improvement." Current opinion in plant biology 10.3 (2007), 317-322.
[8] Porebski, Sue, L. Grant Bailey, and Bernard R. Baum. "Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components." Plant molecular biology reporter 15.1 (1997), 8-15.
[9] Mullis, K.B., Faloona, F., Scharf, S.J., Saiki, R.K., Horn, G.T. and Erlkh, H.A. Specific enzymatic amplification of DNA in- vitro: the polymerase chain reaction. In Cold Spring Harbor symposia on quantitative biology, 51 (1986), 263-273.
[10] Crouch, J. H., et al. "Comparison of PCR-based molecular marker analyses of Musa breeding populations." Molecular Breeding 5.3 (1999), 233-244.
[11] Daniells, Jeff. Musalogue: a catalogue of Musa germplasm: diversity in the genus Musa. Bioversity International, 2001.
[12] Krikorian, A. D. "In vitro culture of bananas and plantains: background, update and call for information." Trop. Agric.(Trin.) 66 (1989), 194-200.
[13] Vuylsteke, D., Swennen, R., Wilson, G.F. and De Langhe. "Phenotypic variation among in-vitro propagated plantain (Musa sp. cultivar 'AAB')." Scientia Horticulturae 36.1-2 (1988), 79-88.
[14] Gupta, P. K., and R. K. Varshney. "The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat." Euphytica 113.3 (2000), 163-185.
[15] Banerjee, N., Vuylsteke, D. and De Langhe, E. Meristem tip culture of musa in : withers LA and Anderson PS (eds.) Butterworths, London (1986), 139-147.
[16] Smith, M. K., and S. D. Hamill. "Early detection of dwarf off-types from micropropagated Cavendish bananas." Australian Journal of Experimental Agriculture 33.5 (1993), 639-644.
[17] Zaffari, G.R., Pereira Pe RFS, L.F., Fernando, Gilbert, B.K. "Indole-3-acetic acid metabolism in normal and dwarf micropropagated banana plants (Musa spp. AAA)." Brazilian Journal of Plant Physiology 14 (2002), 211-217.
[18] Martin, K. P., Suneesh Kumar, P., Zhang, L.L., Slater, A. and Joseph, M. "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 (2006), 188-192.
[19] Ray, T., Dutta, I., Saha, P., Das, S., Roy, S. C. "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 (2006), 11-21.
[20] Sandoval, J. and Muller, L. Considerations la conservation in vitro de Musaceae, possibilities and limitations. Association Bananera National (ASBANA) Costarico, 31, (1989), 21-24.
[21] Cote, F. X., Sandoval, J. A., Marie, P., Auboiron, E. Variations in micropropagated bananas and plantains: literature survey. Fruits, 48.1, (1993), 15-23.
[22] Rajendra Singh, Jayant Nagappan, Soon-Guan Tan, Jothi Malar Panandam and Suan-Choo Cheah. Development of SSR markers for oil palm and finger printing of tissue culture clones. Asia Paci. J. Mole. Bio. & Biotech. 15 (2007), 121-131.
[23] CIRAD. Centre de co-operation internationale recherché agronomique pour i.e., development. http://www.cirad.fr/en/actualite/communique. php. (2003).
[24] Rahman, M., Rajora, O. Microsatellite DNA somaclonal variation in micropropagated Populus tremuloides. Plant Cell Reports. 20 (2001), 531-536.
[25] Goto, S., Thakur, R.C. and Ishii, K. Determination of genetic stability in long term micropropagated shoots of Pinus thunburgii. Plant Cell Reports, 18 (1998), 193-197.
[26] Crouch, J.H., Jaet, E.C., Creyan, P.B. Segregation of microsatellites loci in haploid diploid gametes of Musa. Crop. Si. 38 (1998), 211-217.
[27] Singh, R., Nagappan, J., Rahman, R. A., Tan, S. G., Panandam, J. M., Cheah, S. C., & Board, M. C. 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, (2007), 3-4.
[28] Palambi, M., Damiano, C. Comparison between RAPD and SSR molecular markers in detecting genetic variation in Kiwi fruit. Plant Cell Reports, 20 (2002), 1061-1066.
[29] Hollingsworth, W.O., Christie, C.B., Nichols, M.A., Neilson, H.H. Detection of variation among and within asparagus hybrids using RAPD markers. Newzealand. J. Crop. Horti. Sci. ab. (1997), H97-17.
[30] Rodriguez Lopez, C.M., Wetten, C.A. and Michael, J. Wilkinson, Detection and quantification of in-vitro culture induced chimerism using SSR analysis in Theo broma cacao. TAG Theoretical and Applied Genetics, 110 (2004), 157-166.

DOI: 10.17148/IARJSET.2022.9427

[31] Rani, V., Raina, S. N. Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. In-Vitro Cellular & Developmental Biology-Plant, 36.5 (2000), 319-330.

[32] Jin, S., Mushke, R., Zhu, H., Tu, L., Lin, Z., Zhang, Y., & Zhang, X. Detection of somaclonal variation of cotton (Gossypium hirsutum) using cytogenetics, flow cytometry and molecular markers. Plant Cell Reports, 27.8 (2008), 1303-1316.