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Research article

RAPD MARKER BASED DNA FINGERPRINTING IN RELEASED VARIETIES AND SELECTED SUPERIOR SOMACLONES OF GINGER (*ZINGIBER OFFICINALE* ROSC.)

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ABSTRACT: Random Amplified Polymorphic DNA (RAPD) assay was performed to fingerprint ginger varieties from Kerala Agricultural University "Athira", "Karthika" and "Aswathi" and three selected superior somaclones of ginger viz., B3, 478R and 88R and source parent cultivars Maran and Rio-de-Janeiro. Good quality genomic DNA was extracted from ginger varieties / somaclones and source parent cultivars using CTAB method. Thirty five RAPD primers were screened for amplification of genomic DNA and ten primers were selected based on the amplification pattern. DNA fingerprints of the varieties / somaclones were developed utilizing the clear, distinct bands generated in RAPD profiles and size of the amplicons. Different colour codes were assigned for amplicons produced by the marker system in different varieties, somaclones and source parent cultivars to generate fingerprints. The fingerprints developed were unique and specific for the varieties / somaclones and source parent cultivars.

Key Words: RAPD, Ginger, DNA fingerprinting

INTRODUCTION

India, 'the spice bowl of the world,' enjoys a unique position in the production and export of ginger (*Zingiber officinale* Rosc.) from time immemorial. The crop is much valued as a spice, medicine and vegetable. Breeding of ginger through selection and hybridization is seriously handicapped by lack of variability, absence of natural seed set and exclusive vegetative propagation. As natural variability stands limited, broadening the genetic base through mutagenesis or tissue culture techniques pave way for exploitation of induced variability for isolation of plant types with desirable traits. Somaclonal variation was induced in the two cultivars viz., Maran and Rio-de-Janeiro at College of Horticulture, Kerala Agricultural University through various modes of regeneration and through *in vitro* mutagenesis. The somaclones were subjected to single plant evaluation and they were further evaluated for yield, quality and reaction to pests and diseases [15, 16, 17, 18]. After conducting Initial Evaluation Trials, Advanced Varietal Trials, On Farm Evaluation Trials and Multi Locational Trials, the three superior somaclones, were released as varieties under the names "Athira" "Karthika" and "Aswathi" [25] and somaclones viz., 478R, 88R and B3 were selected as superior clones after the evaluation. The varieties Athira, Karthika and somaclone B3 were derived from the cultivar Maran and the variety Aswathi, somaclones 478R and 88R were from the cultivar Rio-de-Janeiro. DNA fingerprinting is an efficient tool for genotype identification, assessing genetic diversity and protecting plant varieties. DNA fingerprinting was also attempted in ginger by several workers [6, 11, 12, 19, 22]. The RAPD markers are reported to be more suitable for genetic diversity analysis of clonal organisms [3]. Central Seed Committee established under the Seed Act, 1996 stipulates the necessity of DNA fingerprint data for the varieties released or proposed to be released. For the newly released ginger varieties and selected superior somaclones in pipeline for release, no fingerprint data are available. The specific fingerprint data will serve as a mark for identifying the varieties / clones and could be utilized for registration and documentation of varieties, settling IPR issues and to avoid biopiracy.

MATERIALS AND METHODS

Genomic DNA extraction

Three KAU released varieties of ginger (Athira, Karthika and Aswathi), three superior selected somaclones (478R, 88R and B3) and two source parent cultivars (Maran and Rio-de-Janeiro) maintained at Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara were used for the study. Good quality genomic DNA was extracted from one gram young leaf of the ginger varieties/ somaclones/ source parent cultivars following CTAB method reported by Rogers and Bendich [20] and quantified in 0.8% agarose according to a standard DNA marker.

PCR amplification and fragment analysis: RAPD PCR reactions were performed as per the procedure reported by Williams *et al*., [27]. Random decamer primers supplied by Operon Technologies Inc. (Alamedda, Calif.) and Xcelris Genomics Primex with good resolving power were used for amplification of DNA. After initial screening of primers, the decamer primers for RAPD assay were selected. Thirty five primers (Operon Technologies Inc. and Xcelris Genomics Primex) in the series OPA, OPC, OPD, OPE, OPG, OPK, OPP, OPU, OPAH, RN, RY and S (Table 1) were screened with bulked DNA samples from the variety Athira and somaclones 292R and 478R. Genomic DNA at the concentration of 50ng/µl was subjected to amplification using selected random primers. The amplification was carried out in a Veriti^{R} Thermal cycler (Applied Biosystems). The PCR reaction was performed using a 20µl reaction mixture containing 30ng of genomic DNA, 10 X assay buffer (B) (pH 8.3), 10mM/ml dNTP mix, decamer primers, 2mM of MgCl2 and 0.5 U of *Taq (Thermophilus aquaticus)* DNA polymerase (Genei). The basic PCR program to amplify DNA was as follows: an initial hot start and denaturing step at 94°C for 4 min followed by 35 cycles of a 45 seconds denaturation at 94°C, 1 min annealing at 37°C and 2 min primer elongation at 72°C. A final extension step at 72°C for 8 min was performed.

Gel electrophoresis: 15 µl aliquot of the PCR amplified samples was combined with 2µl of a loading buffer (0.4% Bromo-phenol Blue, 0.4% xylene cyanole and 5 ml of glycerol). The amplified products were loaded on two per cent agarose gel using buffer stained with ethidium bromide along with marker (100bp Invitrogen). The documented RAPD profiles were carefully examined for amplification of DNA as bands. The size of polymorphic bands in kb / bp of bases were recorded in comparison with marker and using the software Quantity One.

Out of 35 decamer primers screened for RAPD analysis, primers which gave good amplification products for each ginger variety / clone were selected for further assay.

Data Analysis: Scoring of bands on agarose gel was done with the Quantity One software. λDNA marker (*Eco*RI+*Hind* III double digest, 1000bp) and 100bp ladder were used as molecular weight size marker for each gel along with DNA samples. The bands were scored as one and zero for the presence and absence respectively and their size recorded in relation to the molecular weight markers used and with the software Quantity One.

Generation of fingerprints: DNA fingerprint of each genotype was generated based on the presence of clear and distinct bands and size of the bands. Separate colour codes were given to highlight the presence of unique bands, bands shared with two genotypes, three genotypes etc. In fingerprints generated the presence of unique band was represented in violet colour. Navy blue was used to highlight the bands shared with two genotypes, light blue for bands shared with three genotypes, pink for bands shared with four genotypes, yellow for bands shared with five genotypes, orange was for bands shared with six genotypes, red for bands shared with seven genotypes and green for bands present in all the eight genotypes

RESULTS

The amplification pattern produced by thirty five primers belonging to different RAPD primer series viz., OPA, OPC, OPD, OPE, OPG, OPK, OPP, OPU, OPAH, RN, RY and S are provided in Table 2. Based on the presence of clear and distinct bands, ten decamer primers were selected for RAPD assay of ginger varieties / somaclones which are OPA-04, OPA -12, OPA-27, OPA-28, OPD-15, OPD-20, OPP-16, OPU-03, RN-08 and S-11 (Table 3). Finally the analysis of RAPD profiles was carried out using NTSYS.

DNA fingerprinting of the ginger genotypes

Variety Athira

The amplification pattern observed for the genomic DNA of ginger variety Athira with ten selected RAPD primers is presented in Plate 1A. The number of clear and distinct bands produced by ten RAPD primers was twenty eight.

The size of amplicons ranged from 400bp to 1300bp. Primers OPA 04, OPA 27, OPP 16 and S 11 gave three distinct bands, while it was only one for OPA 28 and OPU 03. The primers OPD 20 and RN 08 gave five distinct and clear bands. Primers OPA 12 and OPD 15 gave two distinct and clear bands. Fingerprint developed based on presence of clear and distinct bands and size of bands produced with ten RAPD primers is presented in Fig. 1A.

Variety Karthika

The amplification pattern observed for the genomic DNA of ginger variety Karthika with ten selected RAPD primers is presented in Plate 1B. Twenty nine clear distinct loci were observed with ten RAPD primers. The amplicons ranged in size 400bp to 2000bp. Primers OPA 12 and OPA 27 gave two and four clear and distinct bands respectively. Primer OPA 04, OPP 16 and S 11 gave three distinct and clear bands, whereas, primers OPA 27 and OPD 20 gave four distinct and clear bands. Only one distinct band was produced by primers OPA 28 and OPU 03 each. Primer OPD 15 gave two distinct bands and RN 08 gave five distinct bands. Fingerprint developed based on clear and distinct bands and size of bands produced with ten RAPD primers is presented in Fig. 1B. The fingerprint revealed that a unique band was developed for the variety Karthika with the random primer OPA 12 of size 2000bp.

Somaclone B3

The amplification pattern observed for the genomic DNA of ginger somaclone B3 with ten selected RAPD primers is presented in Plate 1C. Ten RAPD primers produced twenty six clear and distinct loci. The amplicons ranged in size 400bp to 1300bp. The primers OPA 04, OPA 12 and OPD 15 gave two distinct and clear bands whereas primers OPA 27, OPP 16 and S 11 gave three distinct and clear bands. Primers OPA 28 and OPU 03 gave only one distinct band. Primer OPD 20 gave four distinct bands and primer RN 08 gave five distinct bands. Fingerprint was developed based on clear and distinct bands and size of the bands produced by ten RAPD primers and is presented in Fig. 1C. The fingerprint generated with RAPD profile for somaclone B3 showed a unique band of size 1400bp with the random primer OPA 12.

Cultivar Maran

The amplification pattern observed for the genomic DNA of ginger source parent cultivar Maran with ten selected RAPD primers is presented in Plate 1D. Twenty six clear distinct loci were observed with the ten RAPD primers. The amplicons ranged in size 400bp to 1300bp. Primers OPA 04, OPD 20, OPP 16 and S11 gave three distinct and clear bands, whereas, primers OPA 28 and OPU 03 gave only one clear and distinct band. Primers OPA 12 and OPD 15 gave two bands and primers OPA 27 and RN 08 gave four clear and distinct bands. Fingerprint was developed based on clear and distinct bands produced with the ten selected RAPD primers (Fig. 1D). In the fingerprint generated with RAPD profile, all the bands were shared with the ginger varieties / somaclones studied in the present investigations.

Variety Aswathi

The amplification pattern produced for the genomic DNA of ginger variety Aswathi with ten selected RAPD primers is presented in Plate 1E.

The ten RAPD primers generated twenty six clear distinct loci. The amplicons ranged in size 400bp to 1300bp. Primers OPA 12, OPD 15 and OPD 20 gave two clear and distinct bands, whereas, primers OPA 28 and OPU 03 produced only one clear and distinct band. Primers OPA 04 and OPA 27 produced three distinct and clear bands and primers OPP 16, S 11 and RN 08 gave four distinct and clear bands. Fingerprint developed based on clear and distinct bands produced with selected RAPD primers is presented in Fig 1E. The fingerprint generated by the variety Aswathi with RAPD profile revealed that a unique band of 400bp was produced by random primer OPA 04 for variety Aswathi.

Somaclone 478R

The amplification pattern observed for the genomic DNA of ginger somaclone 478R with ten selected RAPD primers is presented in Plate 1F. The total number of distinct loci observed with ten selected RAPD primers was twenty six. The amplicons ranged in size 400bp to 1300bp (Table 25). Primers OPA 12, OPD 15 and OPD 20 gave two clear and distinct bands, whereas, primers OPA 28 and OPU 03 produced only one clear and distinct band. Primers OPA 04 and OPA 27 produced three distinct and clear bands and primers OPP 16, S 11 and RN 08 gave four distinct and clear bands. Fingerprint was developed based on clear and distinct bands produced by ten selected RAPD primers (Fig. 1F). The salient feature of the fingerprint developed by RAPD profile in somaclone 478R was the unique band of size 300b with the primer OPA 28.

Somaclone 88R

The amplification pattern observed for the genomic DNA of ginger somaclone 88R with ten selected RAPD primers is presented in Plate 1G. A total of twenty six clear distinct loci were observed with the ten RAPD primers.

The amplicons ranged in size 400bp to 1300bp. Primers OPA 12, OPD 15 and OPD 20 gave two clear and distinct bands, whereas, primers OPA 28 and OPU 03 produced only one clear and distinct band. Primers OPA 04 and OPA 27 produced three distinct and clear bands and primers OPP 16, S 11 and RN 08 gave four distinct and clear bands. Fingerprint was developed based on clear and distinct bands produced by ten selected RAPD primers (Fig. 1G).

Cultivar Rio-de-Janeiro

The amplification pattern observed for the genomic DNA of ginger source parent cultivar Rio-de-Janeiro with ten selected RAPD primers is presented in Plate 1H. The ten RAPD primers produced twenty five clear distinct loci. The amplicons ranged in size 400bp to 1300bp. Primers OPA 04, OPA 27 and RN 08 gave three clear and distinct bands, whereas, primers OPA 12, OPD 15 and OPD 20 gave two clear and distinct bands. Primers OPP 16 and S11 gave four clear and distinct bands, whereas, primers OPA 28 and OPU 03 gave only one clear and distinct band. Fingerprint was developed based on clear and distinct bands generated by ten selected RAPD primers (Fig. 1H).

Table 1. RAPD primers used for screening ginger genotypes

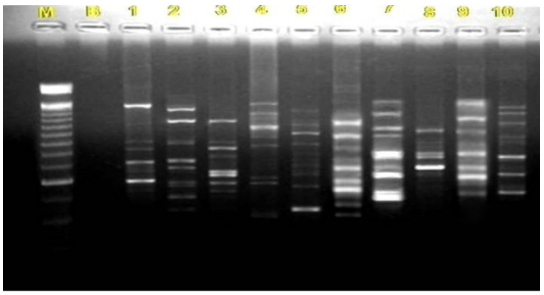
S. No.	Primers	Nucleotide Sequence
1	OPA 02	5’TGCCGAGCTG3’
2	OPA 04	5’AATCGGGCTG3’
3	OPA 06	5’GGTCCCTGAC3’
4	OPA 08	5’GTGACGTAGG3’
5	OPA 10	5’GTGATCGCAG3’
6	OPA 12	5’TGGGCGATAG3’
7	OPA 27	5’GAAACGGGTG3’
8	OPA 28	5’GTGACGTAGG3’
9	OPC 01	5’TTCGAGCCAG3’
10	OPC 02	5’GTGAGGCGTC3’
11	OPC 04	5’GGTACGATGC3’
12	OPC 08	5’TGGACCGGTA3’
13	OPC 14	5’TGCGTGCTTG3’
14	OPD 10	5’GGTCTACCAC3’
15	OPD 15	5’CATCCGTGCT3’
16	OPD 20	5’ACCCGGTAAC3’
17	OPE 05	5’CTGAGAATCC3’
18	OPE 07	5’AGATGCAGCC3’
19	OPG 08	5’TCACGTCCAC3’
20	OPK 01	5’TGGCGACCTG3’
21	OPP 16	5’CCAAGCTGCC3’
22	OPP 17	5’TGACCCGCCT3’
23	OPU 03	5’CTATGCCGAC3’
24	OPU 07	5’CTACAGTGAG3’
25	OPU 13	5’GGCTGGTTCC3’
26	OPAH 1	5’TCCGCAACCA3’
27	OPAH 3	5’GGTTACTGCC3’
28	OPAH 5	5’TTGCAGGCAG3’
29	OPAH 6	5’GTAAGCCCCT3’
30	OPAH 9	5’AGAACCGAGG3’
31	RN 07	5’CAGCCCAGAG3’
32	RN 08	5’ACCTCAGCTC3’
33	RY 08	5’AGGCAGAGCA3’
34	S11	5’GTAGACCCGT3’
35	S 12	5’CCTTGACGCA3’

Table 2. Amplification pattern of RAPD primers in ginger genotypes

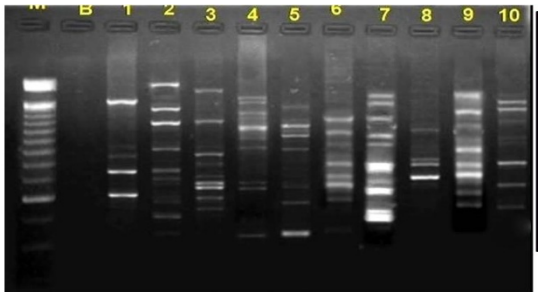
S. No.	Primers	Amplification Pattern			
		No. of bands	Types of bands		Remarks
			Distinct	Faint	
1	OPA 02	9	0	9	------
2	OPA 04	7	3	4	Selected
3	OPA 06	0			------
4	OPA 08	10	3	7	------
5	OPA 10	0			------
6	OPA 12	10	6	8	Selected
7	OPA 27	7	4	3	Selected
8	OPA 28	9	4	5	Selected
9	OPC 01	2	0	2	------
10	OPC 02	3	0	3	------
11	OPC 04	1	0	1	------
12	OPC 08	4	0	4	------
13	OPC 14	4	0	4	------
14	OPD 10	0			------
15	OPD 15	12	5	7	Selected
16	OPD 20	8	8	0	Selected
17	OPE 05	2	0	2	------
18	OPE 07	4	4	0	------
19	OPG 08	0			------
20	OPK 01	2	2	0	------
21	OPP 16	8	4	4	Selected
22	OPP 17	9	2	7	------
23	OPU 03	6	4	2	Selected
24	OPU 07	2	0	2	------
25	OPU 13	1	0	1	------
26	OPAH 1	0			------
27	OPAH 3	9	3	6	------
28	OPAH 5	10	1	9	------
29	OPAH 6	4	1	3	------
30	OPAH 9	6	2	4	------
31	RN 07	11	2	9	------
32	RN 08	11	8	0	Selected
33	RY 08	12	6	6	------
34	S11	8	4	4	Selected
35	S 12	2	0	2	------

Table 3. Selected RAPD primers

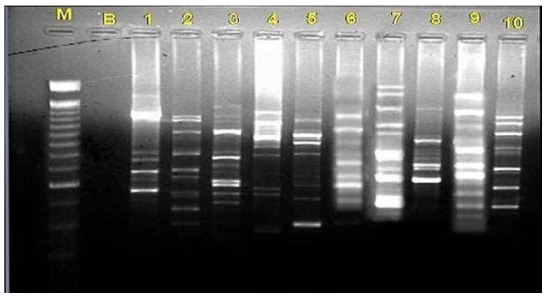
S. No.	Name of Primer	Sequence
1	OPA-04	5’AATCGGGCTG3’
2	OPA -12	5’ TCGGCGATAG3’
3	OPA-27	5’GAAACGGGTG3’
4	OPA-28	5’GTGACGTAGG3’
5	OPD-15	5’CATCCGTGCT3’
6	OPD-20	5’ACCCGGTAAC3’
7	OPP-16	5’CCAAGCTGCC3’
8	OPU-03	5’CTATGCCGAC3’
9	RN-08	5’ACCTCAGCTC3’
10	S-11	5’GTAGACCCGT3’



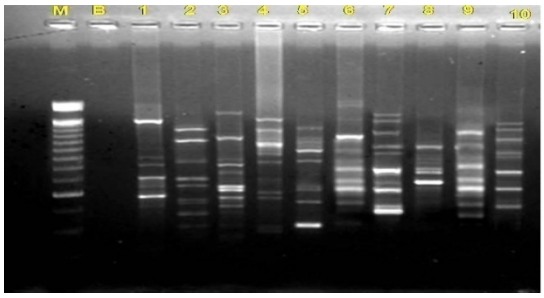
a. Athira



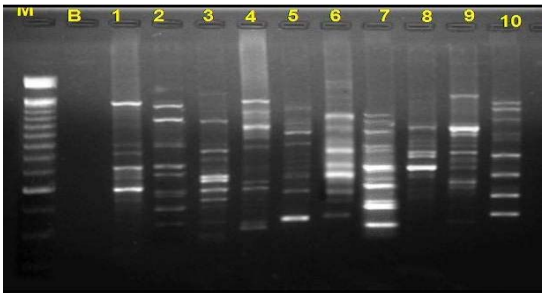
b. Karthika



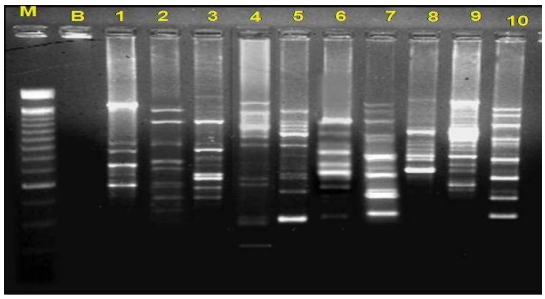
c. B3



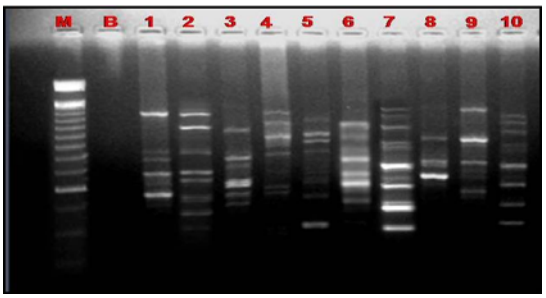
d. Maran



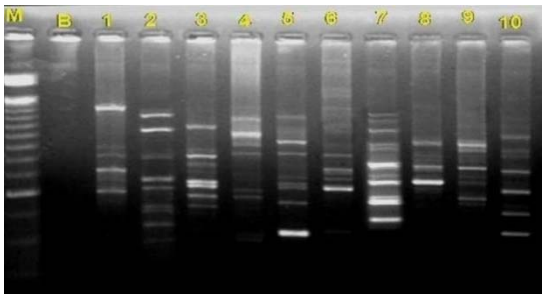
e. Aswathi



f. 478R



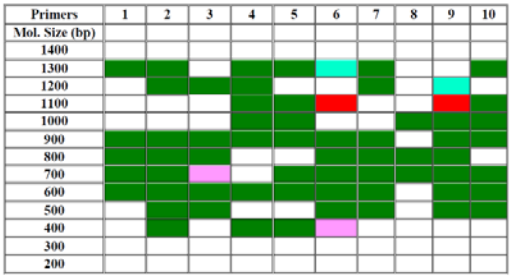
g. 88R



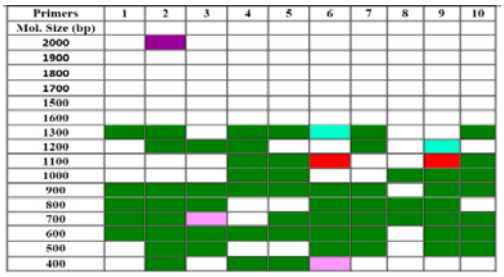
h. Rio-de-Janeiro

M: 100bp ladder, B: Blank
1: OPA 04, 2: OPA 12, 3: OPA 27, 4: OPA 28, 5: OPD 15,
6: OPD 20, 7: OPP 16, 8: OPU 03, 9: RN O8, 10: S 11

Plate 1. Amplification patterns of ginger genotypes with the selected RAPD primers

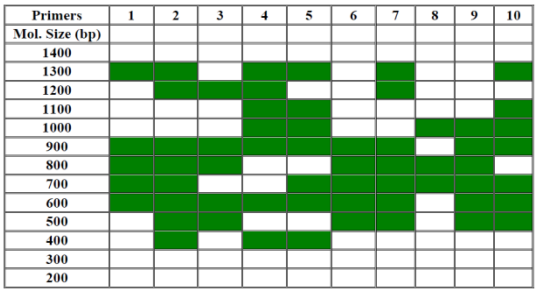


a. Athira

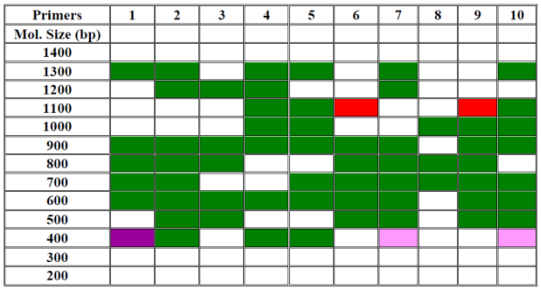


b. Karthika

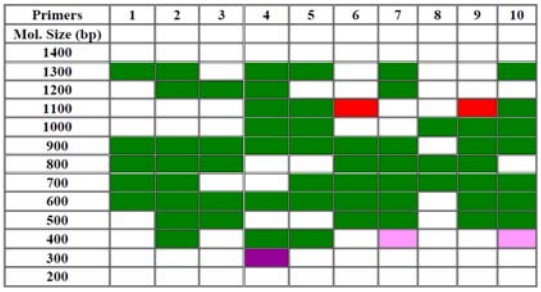
c. B3



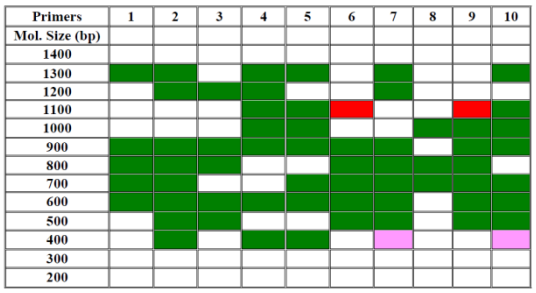
d. Maran



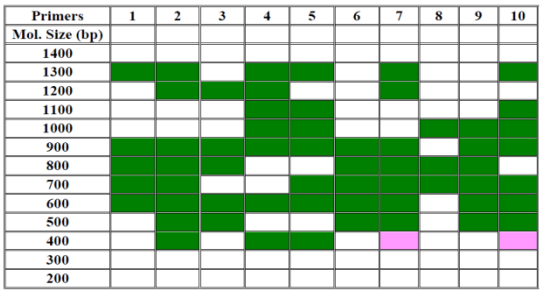
e. Aswathi



f. 478R



g. 88R



h. Rio-de-Janeiro

Colour code for sharing of bands among varieties / somaclones

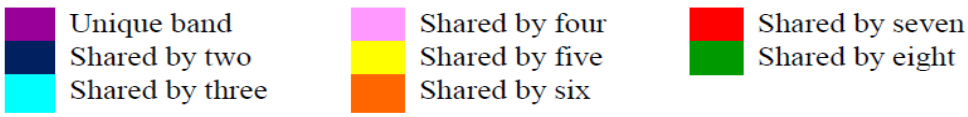


Fig. 1 DNA fingerprints of eight ginger genotypes

DISCUSSION

The RAPD technique was developed by Williams *et al.* [27] and the technique relies on the differential enzymatic amplification of small DNA fragments using PCR with arbitrary decamer primers. In RAPD markers, polymorphism results from the changes in the sequence of the primer binding site. Usually RAPD markers are dominant in nature [26] because polymorphisms are detected as the presence or absence of bands. The RAPD technique [13] is a simple technique which detects polymorphism and has been used for molecular characterization in several crop plants such as *Mangifera indica* L. [1], *Oryza sativa* L. [4], *Piper nigrum* L. [8], *Ficus carica* L. [9], *Manihot esculenta* crantz. [23] and Hybrid rice [24]. The RAPD marker system could bring out unique bands in the variety Karthika and somaclones B3, 292R and 478R with primer OPA 12 in Karthika and B3, OPA 04 in 292R and OPA 28 in 478R. Aptness of RAPD markers in diversity analysis of ginger has been reported in various studies [11, 12, 14, 21]. Therefore, RAPD seems to be a useful tool for identification of ginger genotypes. The variation seen in the varieties / selected somaclones might be due to continuous multiplication during subculturing cycles which might have caused genetic and epigenetic changes to the cultures. The desirable changes occurred were selected during field evaluation trials and they were fixed through vegetative propagation. The genetic and epigenetic changes occurring in the somaclones were reviewed by several workers, [2, 5, 7, 10].

CONCLUSION

The fingerprints generated for the varieties / somaclones from Athira to Rio-de-Janeiro were unique and distinct. Though individual primers showed several bands shared among varieties, somaclones and source parent cultivars, the pattern obtained with all the bands considered together was unique for each genotype which formed the fingerprint of the particular genotype (variety, somaclone or source parent cultivar). The fingerprints thus generated could very well be utilized to prove varietal identity

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