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Molecular characterization of selected somaclones in ginger using RAPD markers

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ABSTRACT

Twelve selected somaclones of ginger cultivars Maran and Rio-de-Janeiro were characterised using RAPD markers. Five primers of the series OPAH and OPP gave good DNA amplification. The primer OPAH 3 exhibited highest polymorphism (33.75%). The molecular characterisation of somaclones revealed the occurrence of genetic variability in the clones evaluated. The extent of genetic variation was more in somaclones of cultivar Maran than clones of cultivar Rio-de-Janeiro. Genetic variation from the source parent cultivar was also more in somaclones of cultivar Maran.

Key words: Diversity, ginger, RAPD, somaclones.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), one of the oldest and renowned spices is esteemed for its aroma, flavour and pungency. It is much valued as a spice, medicine and vegetable since ancient times and used as carminative, stimulant, anti-inflammatory and antiemetic in Ayurvedic system of medicine. Due to exclusive vegetative propagation and lack of natural seed set, natural variability available is very limited in ginger. Broadening the genetic base through *in vitro* mutagenesis or tissue culture techniques is hence of great significance in crop improvement programmes of ginger. Earlier crop improvement research on ginger was mainly focusssed on germplasm collection, evaluation and selection of high yielding clones (Rattan, 11). Induced mutation using γ rays and ethyl methyl sulfonate (EMS) attempted in ginger gave rise to mutants with very low yield and the effects of mutagen treatment vanished in subsequent generations (Dutta and Biswas, 3).

Somaclonal variation often arises in tissue culture as a manifestation of changes in the genome of differentiating vegetative cells induced by tissue culture conditions or epigenetic influence (Larkin and Scowcroft, 6). Any genetic change induced by *in vitro* conditions of tissue culture is expected to generate stable plants carrying interesting heritable traits. Random amplified polymorphic DNA (RAPD) based detection of genetic polymorphism (Williams *et al*., 16) has found successful application in describing somaclonal variation in regenerants of several plant species (Isabel *et al*., 5; Mandal *et al*., 7; Munthali *et al*., 8; Singh *et al*., 14). In the present communication, we report RAPD analysis of selected somaclones of two ginger genotypes namely, Maran and Rio-de-Janeiro.

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MATERIALS AND METHODS

Somaclones of two ginger cultivars derived through direct pathway of bud culture after passing through 10 to 12 *in vitro* subculture cycles and planted out during 1999-2000 for rhizome development, formed the base material for evaluation of yield, quality and reaction to soft rot and bacterial wilt diseases. Conventionally propagated plants of two source parent cultivars Maran and Rio-de-Janeiro served as control. Twelve somaclones (eight somaclones of cultivar Maran and four of cultivar Rio-de-Janeiro), selected based on yield and reaction to soft rot and bacterial wilt diseases selected from 170 somaclones after two years' of field evaluation trials (2002 and 2003) were subjected to RAPD analysis along with source parent cultivars. DNA was extracted from juvenile leaves by Doyle and Doyle (2) method. The quality and quantity of DNA isolated was evaluated by subjecting the DNA to Agarose gel electrophoresis. Genomic DNA of the isolates were amplified using selected random, short oligonucleotide primers. A total of 27 primers (Operon Tech., USA) belonging to OPAH, OPE and OPP series were screened for amplification of genomic DNA. Those primers, which gave maximum number of reproducible bands, were selected and used for further analysis. DNA amplification was performed on a thermal cycler in 25 µl reaction mixture consisting of 2.5 µl 10 X assay buffer with 15 mM $MgCl_2$ (1X), 10 mM dNTPs, 5p moles of single random primer, 20 ng template DNA and 0.6 unit of *Taq* DNA polymerase overlaid with paraffin oil. Reactions were programmed

for one cycle at 94°C for three min., 39 cycles repeated running at 92°C for one min., 37°C for one min. and 72°C for two min. followed by one cycle at 72°C for five min.

The amplification products were resolved by electrophoresis in 1.4 percent agarose gel using 1X TAE buffer and visualized under UV light. The RAPD profiles of the somaclones for different primers were scored based on the presence (1) or absence (0) of bands. Percentage polymorphism for individual primer was calculated as;

$$\text{Percentage polymorphism} = \frac{\text{No. of polymorphic bands}}{\text{Total No. of bands}} \times 100$$

The data were analysed statistically using NTSYS pc 2.0 software programme. The genetic similarity was estimated by Dice's coefficient and dendrogram was constructed employing unweighted pair group method of arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

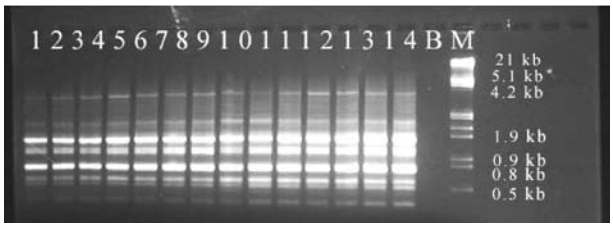
Out of the 27 random primers tested for amplification of DNA, only five primers (OPP 16, OPP 17, OPAH 1, OPAH 3 and OPAH 5) successfully produced good amplification with clear and distinct bands for all the somaclones (Table 1). A total of 494 RAPD band positions were recorded, out of which 154 were polymorphic (Table 2). Percent polymorphism exhibited by primers ranged from zero (OPP 16) to 33.75 per cent (OPAH 3). The primers OPAH 3 and OPAH 1 gave higher polymorphism indicating the presence of heterologous sequences in the genomic DNA (Fig. 1). Amplification by the primer OPP 16 gave no polymorphism indicating the presence of homologous sequences.

Table 1. Primer screening for DNA amplification in ginger.

Sl. No.	Primer code	Primer sequence	No. of bands	Quality of bands	Remarks
1.	OPE 5	GCAGGGAGGT	Zero	No amplification	Rejected
2.	OPE 6	AAGACCCCTC	Zero	No amplification	Rejected
3.	OPE 7	AGATGCAGCC	Two	Clear, less No. of bands	Rejected
4.	OPE 8	TCACCACGGT	Zero	No amplification	Rejected
5.	OPE 9	CTTCACCCGA	Zero	No amplification	Rejected
6.	OPE 10	CACCAGGTGA	Zero	No amplification	Rejected
7.	OPE 11	GAGTCTCAGG	Zero	No amplification	Rejected
8.	OPE 12	TTATCGCCCC	Zero	No amplification	Rejected
9.	OPP 1	GTAGCACTCC	Zero	No amplification	Rejected
10.	OPP 2	TCGGCACGCA	Zero	No amplification	Rejected
11.	OPP 3	CTGATACGCC	Zero	No amplification	Rejected
12.	OPP 4	GTGTCTCAGG	Zero	No amplification	Rejected
13.	OPP 5	CCCCGGTAAC	Zero	No amplification	Rejected
14.	OPP 6	GTGGGCTGAC	One	Smeared	Rejected
15.	OPP 7	GTCCATGCCA	Zero	No amplification	Rejected
16.	OPP 8	ACATCGCCCA	Zero	No amplification	Rejected
17.	OPP 13	GGAGTGCCTC	Zero	No amplification	Rejected
18.	OPP 14	CCAGCCGAAC	Zero	No amplification	Rejected
19.	OPP 15	GGAAGCCAAC	Zero	No amplification	Rejected
20.	OPP 16	CCAAGCTGCC	Four	Clear, distinct	Selected
21.	OPP 17	TGACCCGCCT	Four	Clear, distinct	Selected
22.	OPAH 1	TCCGCAACCA	Four	Clear, distinct	Selected
23.	OPAH 2	CACTTCCGCT	Zero	No amplification	Rejected
24.	OPAH 3	GGTTACTGCC	Eight	Clear, distinct	Selected
25.	OPAH 4	CTCCCCAGAC	Zero	No amplification	Rejected
26.	OPAH 5	TTGCAGGCAG	Eight	Clear, distinct	Selected
27.	OPAH 6	GGCTTGGCCT	Zero	No amplification	Rejected

Table 2. RAPD polymorphism in selected primers in ginger.

Primer	Total No. of bands	No. of polymorphic bands	% polymorphism
OPP 16	112	0.00	0.00
OPP 17	103	19.00	18.45
OPAH 1	96	22.00	22.92
OPAH 3	80	27.00	33.75
OPAH 5	103	18.00	17.48



OPP 17

Fig. 1. RAPD profile of selected somaclones in ginger. (1. 281 R, 2. 386 R, 3. 364 R, 4. 292 R, 5. M VI, 6. 342 M, 7. 100 M, 8. 393 M, 9. 918 M, 10. 970 M, 11. 99 M, 12. 488 M, 13. R, 14. M)

Analysis of genetic variability among the different somaclones indicated that genetic variability existed within and between somaclones of two cultivars and source parent plants. The percentage of genetic variability varied from zero to 31.6 per cent in somaclones of cultivar Maran with an average variability of 16.8 per cent (Table 3). Highest genetic dissimilarity of 31.6 per cent was observed between two somaclones, *viz*., M VI and 488 M followed by M VI and 342 M and 488 M and 342 M with 24.4 per cent variability. The highest genetic similarity of 100 per cent was observed between somaclones 393 M and 918 M. The genetic dissimilarity between the source parent plant (M) and the somaclones ranged between 2.5 per cent (342 M) to 22.5 per cent (M VI and 488 M). Evidently, M VI had accumulated maximum genetic changes and had the highest genetic variability with the source parent plant (M) and rest of the somaclones. Followed by M VI, the clone 488 M exhibited high genetic variability with the source parent plant (M) and rest of the somaclones.

The genetic variability in somaclones of Rio-de-Janeiro varied between 2.8 to 20 per cent with an average variability of 10.8 per cent (Table 4). Highest variability of 20 per cent was observed between 292 R and 281 R and lowest variability of 2.8 per cent was observed between 364 R and 386 R. The clone 281 R showed highest dissimilarity with the source parent plant (R) (12.8%). The somaclone 292 R was totally distinct from other somaclones and the source parent plant (R). Rout *et al.* (12) in ginger, Salvi *et al.* (13) and Panda *et al.* (9) in turmeric observed no RAPD polymorphism between somaclones and source parent plant. The present study in ginger revealed that *in vitro* conditions have induced varied amount of genetic variation in the somaclones analysed. Bin *et al*. (1) in amorphophallus, Han *et al*. (4) in sweet potato and Prakash *et al*. (10) in mango ginger reported polymorphism in RAPD banding pattern of somaclones. Cultivar difference was observed in the extent of genetic variation. The variation was more in somaclones of cultivar Maran (0-31.6 %) with an average variability of 16.8 per cent. Somaclones

Table 3. Matrix of dissimilarity in selected somaclones of ginger cultivar Maran.

	M VI	342 M	100 M	393 M	918 M	970 M	99 M	488 M	Control M
M VI	0.000								
342 M	0.244	0.000							
100 M	0.220	0.024	0.000						
393 M	0.243	0.175	0.195	0.000					
918 M	0.243	0.175	0.195	0.000	0.000				
970 M	0.184	0.075	0.098	0.158	0.158	0.000			
99 M	0.158	0.098	0.073	0.179	0.179	0.026	0.000		
488 M	0.316	0.244	0.220	0.194	0.194	0.231	0.205	0.000	
Control M	0.225	0.025	0.049	0.154	0.154	0.051	0.075	0.225	0.000

Table 4. Matrix of dissimilarity in selected somaclones of ginger cultivar Rio-de-Janeiro.

	281 R	386 R	364 R	292 R	Control R
281 R	0.000				
386 R	0.083	0.000			
364 R	0.108	0.028	0.000		
292 R	0.200	0.128	0.103	0.000	
Control R	0.128	0.103	0.077	0.122	0.000

of cultivar Rio-de-Janeiro exhibited 2.8 to 20 per cent variability with an average variability of 10.8 per cent. The extent of genetic variation from the source parent plant was more in somaclones of cultivar Maran (12%) than in clones of cultivar Rio-de-Janeiro (10.8%).

The dendrogram constructed using pooled data of RAPD analysis with five different primers expressed the total genetic makeup of the somaclones and source parent plants. The *in vitro* regeneration process is mainly responsible for the difference in RAPD banding pattern in the somaclones (Soniya *et al*., 15). The somaclones of Maran had two clusters based on the dendrogram (Fig. 2). The first cluster consisted of M VI alone showing its distinctness from rest of the somaclones and the source parent cultivar (M). Within the second cluster, 488 M differed from rest of the somaclones. The clones 393 M and 918 M shared maximum similarity with each other followed by the clones 342 M and 100 M and 970 M and 99 M. The clones 342 M and 100 M shared maximum similarity with the source parent cultivar (M).

The dendrogram constructed for somaclones of cultivar Rio-de-Janeiro formed two clusters (Fig. 3). The first cluster consisted of 292 R alone showing its distinctness from rest of the somaclones and the original parent cultivar (R). In the second cluster, 281 R differed from rest of the somaclones. The clones 386 R and 364 R shared maximum similarity with each other and the parent cultivar plant (R).

RAPD analysis revealed genetic variability within and between somaclones of two cultivars and somaclones and source parent cultivars. In the somaclones of cultivar Maran analysed, M VI exhibited

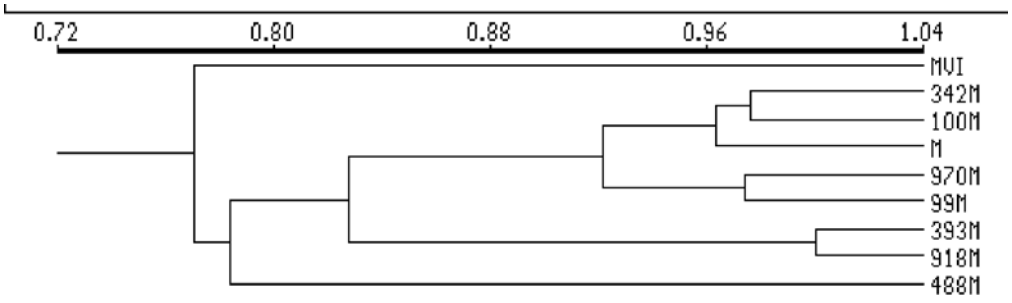


Fig. 2. Dendrogram of RAPD profiles of somaclones in ginger cultivar Maran.

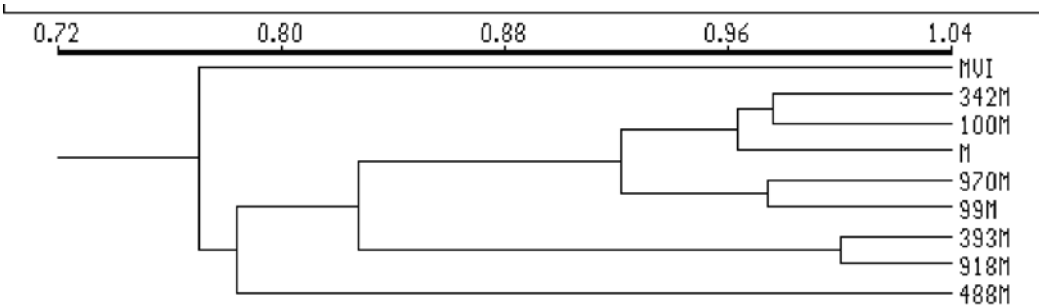


Fig. 3. Dendrogram of RAPD profiles of somaclones in ginger cultivar Rio-de-Janeiro.

highest polymorphism from rest of the somaclones of the cultivar and source parent. In somaclones of cultivar Rio-de-Janeiro, the clone 292 R differed from rest of the somaclones of the cultivar and the source parent. The genetic variability was high in somaclones of cultivar Maran as compared to cultivar Rio-de-Janeiro. Molecular characterization of selected somaclones using RAPD analysis revealed the genetic variation in somaclones. Hence, *in vitro* culture induced variation could be utilized for creation of variability for crop improvement in ginger.

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