

Original Research Article

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## Variability Analysis in Ginger (*Zingiber officinale* Rosc.) Somaclones Using RAPD Marker System

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### ABSTRACT

#### Keywords

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Thirteen groups of somaclones (seven in Maran and six in Rio-de-Janeiro) were evaluated using Random Amplified Polymorphic DNA (RAPD). RAPD analysis using twelve selected primers produced 129 amplicons, 44 were polymorphic with an average of 3.66 polymorphic bands / primer and a polymorphism percentage of 34.10. The dendrograms generated based on RAPD profiles grouped the somaclones into two separate clusters. RAPD marker system showed that somaclones derived from cultivar Maran exhibited more variability than Rio-de-Janeiro. In group wise variability analysis using bulked DNA, the groups RC20 Gy and RSe10 Gy recorded higher variability from source parent cultivar. The variability exhibited in plant wise analysis using two selected primers was found very high (39%) as compared to group wise analysis (25%). The somaclone RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed more variability exhibiting 59 and 53 per cent respectively from the source parent cultivar Rio-de-Janeiro.

### Introduction

Ginger (*Zingiber officinale* Rosc.), an important spice crop grown in India, is much valued for its flavour and medicinal properties. Crop improvement through selection and hybridization are not effective in ginger due to lack of variability and absence of natural seed set. The earlier crop improvement programmes were hence focussed on mutation breeding using  $\gamma$  rays and ethyl methyl sulfonate (EMS). The mutants thus isolated were low yielders and the effect of mutagen treatment vanished in subsequent generations (Giridharan, 1984;

Dutta and Biswas, 1985; Jayachandran 1989). Hence investigations were made to induce variability in ginger through biotechnological tools like *in vitro* pollination and exploitation of somaclonal variation.

Currently, molecular marker techniques are widely employed to detect and assess somaclonal variation in several crops as they are stable, detectable in all tissues and are not confounded by environment, pleiotropic and epistatic effects. RAPD markers could detect somaclonal variation in beet (Munthali *et al.*, 1996), garlic (Zahim *et al.*, 1996), date palm (Saker *et al.*, 2000), banana (Gimenez *et al.*,

2001 and Mohamed, 2007), tomato (Soniya *et al.*, 2001) and potato (Ehsanpour *et al.*, 2007) in varying degrees. The variability analysis in the thirteen groups of ginger somaclones (180 Nos.) of two cultivars Maran and Rio-de-Janeiro using RAPD marker system was attempted in the present study.

## Materials and Methods

The somaclones from bud culture of the two cultivars Maran and Rio-de-Janeiro were planted out for rhizome formation in 1999-2000 after passing through ten to twelve subculture cycles and the clones were evaluated for yield, quality and tolerance to soft rot and bacterial wilt diseases. Preliminary field evaluation, advanced variety trials, on farm evaluation multilocational tests, large scale demonstration of selected clones were undertaken during the period from 2002 to 2010 and two selected superior somaclones from bud culture regenerants were released under the name Athira and Karthika during 2010. The somaclones regenerated through indirect methods and regenerants from irradiated organogenic and embryogenic calli of the two cultivars were planted out for rhizome formation in 2004. Preliminary yield evaluation and evaluation for soft rot and bacterial wilt diseases in the clones were completed during 2006 to 2010. The evaluation of the clones at molecular level and variability analysis using RAPD was attempted in the present study. Thirteen groups of somaclones which include 180 plants in total (seven groups in Maran and six groups in Rio-de-Janeiro) based on mode of regeneration along with two source parent cultivars were subjected to RAPD analysis.

DNA was extracted from somaclones using CTAB method (Rogers and Bendich, 1994) and Sigma's GenElute™ Plant Genomic DNA Miniprep kit. The quality and quantity of genomic DNA was estimated using

NanoDrop<sup>R</sup> ND-1000 spectrophotometer and agarose gel electrophoresis. The somaclones were grouped as per genotype and mode of regeneration. DNA extracted from individual 180 somaclones was bulked as per the procedure reported by Dulson *et al.*, (1998). Bulk DNA samples of the thirteen groups of somaclones (seven in Maran and six in Rio-de-Janeiro) along with two source parent cultivars were amplified using selected RAPD primers. The genotype and mode of regeneration exhibiting more variability was focused for further in depth investigations, using individual DNA of each somaclone.

A total of 35 decamer primers were screened and those primers which gave good amplification products were selected for further analysis. The DNA amplification was performed in a thermal cycler in 20 µl reaction mixture consisting of 2 µl 10X *Taq* assay buffer B with 2 µl MgCl<sub>2</sub>, 1.5 µl dNTPs, 10pM of single random primer, 30 ng template DNA and 0.4 µl of *Taq* DNA polymerase. Reactions were programmed for one cycle at 93°C for one min., 40 cycles repeated running at 93°C for one min., 37°C for one min. and 72°C for two min. followed by one cycle at 72°C for eight min. The amplification products were resolved by electrophoresis in two percent agarose gel using 1X TAE buffer and visualized under UV light. The RAPD profiles of the thirteen groups of somaclones for different primers were scored based on the presence (1) or absence (0) of bands. The data were analysed using NTSYS pc version 2.02i (Rohlf, 2005). The genetic similarity was estimated by Jaccard's coefficient and dendrogram was generated by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Resolving power (Rp) of a primer was calculated as the sum of 'band informativeness' of all the bands produced by the primer. Band information (Ib) is  $= 1 - [2(0.5 - p)]$ , where p is the proportion of the somaclones containing the bands.

Resolving power of the primer is represented as:  $R_p = \sum I_b$ . PIC value was calculated according to Anderson (1993) as  $PIC = 1 - \sum p_i^2$ , where  $p_i$  was the frequency of the  $i^{th}$  allele.

## Results and Discussion

### Variability analysis in ginger somaclones with selected RAPD primers

Out of the 35 RAPD primers screened, twelve gave good amplification (Table 1). RAPD analysis using twelve selected primers produced 129 amplicons, 44 were polymorphic with an average of 3.66 polymorphic bands / primer and a polymorphism percentage of 34.10 (Table 2). The polymorphism percentage ranged from 10 to 54.54 in the selected RAPD primers. The highest polymorphism percentage was recorded by the primer OPA 28 (54.54) followed by OPD 15 (45.45) and S11 (45.45). The primer OPA 27 recorded lowest polymorphism percentage (10). The study could identify certain specific RAPD primers for identification of Maran and Rio-de-Janeiro cultivars and also irradiated mutants from non-irradiated somaclones. The RAPD primers OPA 12, OPA 27, OPU 03, S11 and OPAH 03 could be utilized for identification of Maran and Rio-de-Janeiro cultivars.

The polymorphism information content (PIC) of selected primers ranged from 0.87 (OPA 04) to 0.92 (RN 08 and OPAH 03) with a mean of 0.90. The Resolving power ( $R_p$ ) calculated for the twelve random primer ranged between 15.5 (OPA 04) and 23.3 (OPAH 03) with an average of 19.98 for RAPD primers.

The dendrogram generated using NTSYS grouped the somaclones into two main clusters. Cluster I includes eleven groups of somaclones (MB, MC, MSe, MC10 Gy,

MC20 Gy, MSe10 Gy, MSe20 Gy, RB, RC, RSe, RC10 Gy) and two source parent cultivars. Rio-de-Janeiro regenerants from calli irradiated with 20 Gy and somatic embryo regenerants from embryogenic calli irradiated with 10 Gy formed the second main cluster. Cluster I was divided into two major sub clusters, with somaclones of Maran in first subcluster and somaclones of Rio-de-Janeiro in second subcluster (Figure 1). A genetic similarity matrix of different groups of ginger somaclones based on the proportion of shared RAPD fragments was also generated. The pairwise similarity coefficient values varied between 0.6589 and 1.0000 indicating 34 per cent variability in somaclones and induced mutants of different groups. Sajeeva *et al.*, (2011) reported a Jaccard's similarity coefficient of 0.57-0.96 when they assessed variability in ginger germplasm.

The extent of variability in somaclones from the source parent cultivar Maran was found more in MC20 Gy (5.4%) followed by MSe20 Gy (4%), MSe10 Gy (2.5%), MC10 Gy (2.5%) MB (2.5%) and callus regenerants (2.5%) while somatic embryo regenerants without irradiation showed less variability (1.5%). In Rio-de-Janeiro, the highest variability was recorded by RC20 Gy (28%) followed by RSe10 Gy (22%). However, the indirect organogenic and embryogenic regenerants without irradiation showed less variability (1.1%) as compared to irradiated groups. The somaclone derived from cultivar Maran exhibited more variability than somaclones of Rio-de-Janeiro. Paul *et al.*, (2012) reported the same result when they characterized selected ginger somaclones using RAPD markers.

Molecular data obtained from RAPD marker systems compared between modes of regeneration, genotypes and source parent cultivars. RAPD markers helped to assess the extent of somaclonal variation in ginger at

molecular level as influenced by genotype and mode of regeneration. The Rio-de-Janeiro indirect organogenesis irradiated with 20 Gy and Rio-de-Janeiro indirect embryogenesis irradiated with 10 Gy were found to be more variable among all the groups of ginger somaclones. Hence these two groups were analysed for variability.

RAPD marker system was found effective for bringing out variability in ginger somaclones. Suitability of RAPD markers in diversity analysis of ginger was also reported by Rout *et al.*, (1998), Nayak *et al.*, (2005), Palai and Rout (2007). The somaclone derived from cultivar Maran exhibited more variability than somaclones of Rio-de-Janeiro.

Irradiated callus and somatic embryo regenerants showed more variability.

The group wise variability analysis using bulked DNA gave an indication of the extent of variability of the group from source parent cultivars. The variability exhibited in plant wise analysis of the selected variable groups was found high and two plants with high

variability could be selected. The somaclone RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed higher variability exhibiting 59 and 53 per cent variability respectively from source parent cultivar Rio-de-Janeiro. In crops like ginger where the natural variability is very less, *in vitro* mutagenesis could be employed for widening the genetic base in ginger.

### Variability analysis in ginger somaclones of groups RC20 Gy and RSe10 Gy using two RAPD primers

Initially, amplification of bulked DNA samples from each of the thirteen groups of ginger somaclones and their source parent cultivars was carried out using selected primers of each marker system. Amplification of thirteen groups of ginger somaclones and their source parent cultivars could reveal noticeable variability in RC20 Gy and RSe10 Gy among all the groups of somaclones studied. This may be due to the mode of regeneration and effect of  $\gamma$  irradiation on *in vitro* multiplication.

**Table.1** Details of selected RAPD primers

Sr. No.	Name of Primer	Sequence
1	OPA-02	5'TGCCGAGCTG3'
2	OPA-04	5'AATCGGGCTG3'
3	OPA -12	5' TCGGCGATAG3'
4	OPA-27	5'GAAACGGGTG3'
5	OPA-28	5'GTGACGTAGG3'
6	OPD-15	5'CATCCGTGCT3'
7	OPD-20	5'ACCCGGTAAC3'
8	OPP-16	5'CCAAGCTGCC3'
9	OPU-03	5'CTATGCCGAC3'
10	RN-08	5'ACCTCAGCTC3'
11	S-11	5'GTAGACCCGT3'
12	OPAH-03	5'GGTACTGCC3'

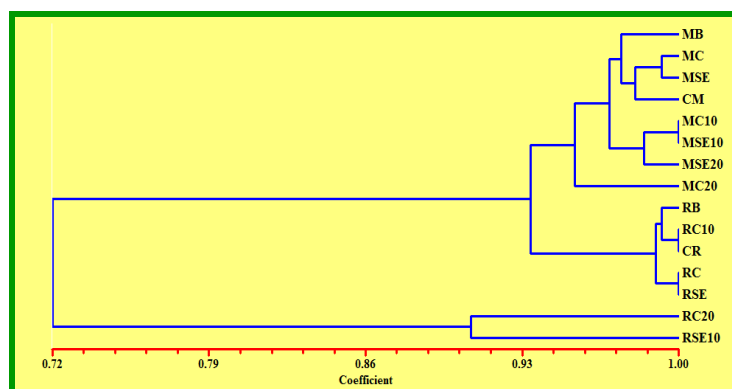
**Table.2** Amplification pattern of selected primers for RAPD assay in ginger

Sr. No.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Size of amplicons (range-bp)	Polymorphism (%)
1	OPA-02	11	4	7	300-1100	36.36
2	OPA-04	8	2	6	150-1500	25
3	OPA -12	12	5	7	300-1600	41.66
4	OPA-27	10	1	9	400-1600	10
5	OPA-28	11	6	5	300-1500	54.54
6	OPD-15	11	5	6	300-1500	45.45
7	OPD-20	10	3	7	200-1800	30
8	OPP-16	10	2	8	400-1400	20
9	OPU-03	10	2	7	400-1500	20
10	RN-08	12	4	8	300-1700	33.33
11	S-11	11	5	6	300-1800	45.45
12	OPAH-03	13	5	8	200-1600	38.46
<b>Total</b>		129	44	84		34.10
<b>Average</b>		10.75	3.66	7		

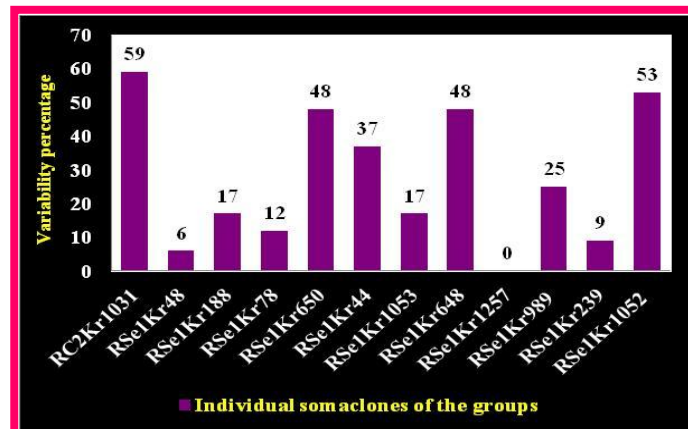
**Table.3** Details of amplification with selected primers in individual ginger somaclones of group RC20 Gy and RSe10 Gy

Sr. No.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Size of amplicons (range-bp)	Polymorphism (%)
1	OPA 28	14	9	5	300-1300	64.28
2	S 11	13	9	4	300-1500	69.23
<b>Total</b>		27	18	9		66.66
<b>Average</b>		13.5	9	4.5		

**Fig.1** Dendrogram generated with RAPD profile in different groups of ginger somaclones



**Fig.2** Variability in individual somaclones of the group RC20 Gy and RSe10 Gy



The group wise variability analysis using bulked DNA gave an indication of the extent of variability of the group from source parent cultivars. Hence RC20 Gy and RSe10 Gy groups were focused for further investigations. DNA from each of these twelve somaclones (one of RC20 Gy and eleven of RSe10 Gy groups), were used for further variability analysis.

Two RAPD (OPA 28 and S11) primers which recorded highest polymorphism were used to amplify the DNA of individual somaclones in RC20 Gy and RSe10 Gy groups along with Rio-de-Janeiro source parent cultivar.

The number of polymorphic amplicons detected by OPA 28 and S 11 primers was 9 and 9 respectively. Hence polymorphism percentage recorded by OPA 28 and S 11 primers was 64.28 and 69.23 per cent respectively (Table 3). Variability exhibited in plant wise analysis using the three selected primers was thus very high (39%) as compared to group wise analysis (25%). Similar observations were reported by Fu *et al.*, (2003) when they assessed effectiveness of several bulking strategies in detecting RAPD variations in flax (*Linum usitatissimum* L.). In the study they observed that about 30 per cent of the polymorphic RAPD loci observed in plant-by-plant analysis were undetected in the bulked samples of the same accession.

Using NTSYS, individual ginger somaclones were grouped into two main clusters. From individual somaclones analysis five somaclones exhibiting more variability from source parent cultivar could be isolated (37% to 59%). Two somaclones RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed more variability exhibiting 59 and 53 per cent variability respectively from source parent cultivar Rio-de-Janeiro (Figure 2).

The mutants which exhibited more variability from source parent cultivar could be further evaluated for their desirable traits. The groups of somaclones which exhibited more variability in the present study were due to the absence of amplicons in the various molecular marker systems analysed. This may be due to the chromosome aberration and rearrangements, DNA methylation or histone modification as explained in somaclones and mutants by several workers (Larkin and Scowcroft, 1981; Skirvin and Janick, 1996; Bairu *et al.*, 2011).

In the crops like ginger where natural variability is less, the present investigation could broaden the genetic base.

From the present investigations the following conclusions could be drawn:

Molecular marker techniques could be employed for the assessing the variability in

ginger somaclones. The variability observed in RAPD assay was 28 per cent

The study could identify certain specific RAPD markers for identification of Maran and

Rio-de-Janeiro cultivars and also irradiated mutants from non-irradiated somaclones.

The somaclone derived from cultivar Maran exhibited more variability than somaclones of Rio-de-Janeiro.

Irradiated callus and somatic embryo regenerants showed more variability

The groupwise variability analysis using bulked DNA gave an indication of the extent of variability of the group from source parent cultivars.

The variability exhibited in plantwise analysis of the selected variable groups was found high and two plants with high variability could be selected.

The somaclone RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed higher variability exhibiting 59 and 53 per cent variability respectively from source parent cultivar Rio-de-Janeiro.

In crops like ginger where the natural variability is very less,

*In vitro* mutagenesis could be employed for widening the genetic base in ginger.

### **Future prospects**

Molecular marker analysis of individual plants of the variable groups,

Use of advanced marker systems for assessment of somaclonal variation,

Use of more number of primers to bring out variability and more focus on *in vitro*

mutagenesis for widening the genetic base in ginger are the future areas to be investigated.

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