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Gene Note

Identification and genetic variation among eight varieties of ginger by using random amplified polymorphic DNA markers

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Abstract Identification and characterization of germplasm is an important link between the conservation and utilization of plant genetic resources. The present investigation was undertaken for identification and genetic variation within eight high yielding varieties of ginger through RAPD markers. A total of 55 distinct DNA fragments ranging from 0.5–2.4 Kb were amplified by using twelve selected primers. The cluster analysis indicated that the eight varieties formed two major clusters.The first major cluster had only one variety 'S-558' with 43% similarity with other seven varieties. Second major cluster having seven varieties and divided into two minor clusters. One minor cluster had six varieties ('Jugijan', 'Turia local', 'Nadia', 'Zo-17', 'Nahfrey' and 'Gurubathan) and other having only one variety ' Surabhi'. The second minor cluster further divided into two sub-minor clusters. 'Nadia' and 'ZO-17' had 78% similarity among themselves and 70% similarity with 'Jugijan' and 'Turia local'. 'Jugijan' and 'Turia local' were having 81% similarity among themselves. However, 'Nahfrey' had 64% similarity with 'Jugijan', 'Turia local', 'Nadia' and 'ZO-17'. The present study showed the distant variation within the varieties. This investigation will help to breeders for ginger improvement program.

Key words: Ginger, genetic variation, RAPD.

Zingiber officinales Rosc. (ginger) of the family Zingiberaceae is an important tropical horticultural plant, values all over the world as a spices and for its medicinal properties. It is rich in secondary metabolite such as Oleoresin (Bhagyalakshmi and Singh, 1988). Breeding of ginger is seriously handicapped by poor flowering and seed set. It is propagated vegetatively through rhizome. The germplasm collections in clonal repositories are also seriously affected by fungal diseases. Taking into account the utility, the conservation of genetic diversity and building up of nuclear base populations is essential for improvement of ginger. The most important role of conservation is to preserve the genetic variation and evolutionary process in viable populations of ecologically and commercially viable varieties/ genotypes in order to prevent potential extinction. Molecular markers have provided a powerful new tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited traits. The molecular approach for identification of plant varieties/genotypes seems to be more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants (Williams et al. 1990, Paterson et al. 1991). PCR-based molecular markers have been widely used in many plant species for identification, phylogenetic analysis, population studies and genetic linkage mapping (Williams et al. 1990). The RAPD markers can also be used in the study of the genetic variability of species or natural populations (Lashermes et al. 1993, Wilkie et al. 1993) and in the identification of genotypes (Wilde et al. 1992, Koller et al. 1993, Wolff and Peters-Van Run 1993). In this communication, we report the identification and genetic variation among eight varieties of ginger by using RAPD markers.

Eight high yielding varieties (*Zingiber officinales* vars. Surabhi, Turia Local, Jugijan, Nadia, S-558, Gurubathan, ZO-17, Nahfrey) of ginger were collected from High Altitude Research Station, Pattangi, Orissa University of Agriculture and Technology, Orissa. The rhizomes were planted in the nursery bed for sprouting. The young leaves were used for DNA analysis.

DNA was extracted from fresh leaves collected from nursery by using the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Approx. 20 mg of fresh leaves was ground to powder in liquid nitrogen using a mortar and pestle. The ground powder was transferred to a 50 ml falcon tube with 10 ml of CTAB buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris (tris(hydroxymethyl) aminomethane)-HCl, pH 8.0, and 0.2% (v/v) α-mercaptoethanol]. The

Abbreviations: PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA
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homogenate was incubated at 60°C for 2 h, extracted with an equal volume of chloroform/isoamyl alcohol (24 : 1 v/v) and centrifuged at 10,000×*g* for 20 min (Kubota KR-2000 C, Rotor-RA-3R, Tokyo, Japan). DNA was precipitated from the aqueous phase by mixing with an equal volume of isopropanol. After centrifugation at 10,000×*g* for 10 min, the DNA pellet was washed with 70% (v/v) ethanol, air-dried and resuspended in TE (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA) buffer. DNA quantifications were performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel. The resuspended DNA was then diluted in TE buffer to 5 μg/μl concentration for use in polymerase chain reaction (PCR).

Forty decamer primers, corresponding to kits A, C, D, and N from Operon Technologies (Alameda, California, USA) were initially screened using one variety of ginger 'Surabhi' to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species/varieties. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

Polymerase chain reactions (PCR) with single primer were carried out in a final volume of 25 μl containing 20 ng template DNA, 100 μM of each deoxyribonucleotide triphosphate, 20 ng of decanucleotide primer (M/S Operon Technology), 1.5 mM $MgCl_2$, 1×Taq buffer [10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.001% gelatin], and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, India). Amplification was performed in a PTC-100 thermal cycler (M J Research Inc., Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94°C, followed by 40 cycles of denaturation at 94°C for 20 s., annealing at 38°C for 30 s and extension at 72°C for 1 min, finally at 72°C for 10 min for RAPD amplification. Amplification products were separated alongside a molecular weight marker (1.0 kb plus ladder, M/S Bangalore Genei) by 1.2% agarose gel electrophoresis in 1×TAE (Tris acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad, USA).

Data were recorded as presence (1) or absence (0) of band products from the photographic examination. Each amplification fragment was named by the source of the primer, the kit letter or number, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity index was estimated using the formula, $S=2\ N_{AB}/N_A+N_B$ (Nei and Li 1979).
Where, N_{AB} is the number of amplified products common to both A and B.

N_A and N_B correspond to number of amplified product in A and B respectively. The average of similarity matrices was used to generate a tree by UPGMA (unweighted pair-group method arithmetic average) using NTSYS-PC, version 2.0 (Rohlf 1995).

The present study offers an optimization of primer screening for evaluation of genetic relationship among eight varieties of *Zingiber officinale* through RAPD analysis. The variety 'Surabhi' was used for screening primers obtained from different series for amplification by using polymerase chain reactions. The results showed that A- and N-series primers produced relatively more amplification fragments compared to other series of primers. The amplification generated by primers OPC-14, OPC-18 and OPC-04 produced small numbers of fragments by using the variety 'Surabhi'. The primers OPN-09, OPN-10 and OPN-15 produced maximum number of DNA fragments; the size of the DNA fragments ranged from 0.5 to 2.0 base pairs. The primer OPA-19 amplified 4 fragments whereas, OPN-10 produced 5 bands in var. Surabhi. It was also noted that some primers did not show any amplification in some of the variety. The number of fragments varied from one series of primers to other series. The twenty-decamer primers produced good amplification of RAPD fragment ranging from 500 to 2400 base pairs. Subsequently, 12 primers were selected and used to analyze the genetic relationship among eight varieties of ginger through polymerase chain reaction. The reproducibility of the amplification product was tested on DNA from three independent extractions of the varieties. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. As a result, twelve informative primers were selected and used to evaluate the degree of polymorphism within eight varieties of ginger. The selected primers generated distinctive products in the range of 0.5 to 2.4 Kb. The maximum and minimum number of bands was produced by the primers OPN-15 (6), OPN-10 (6), OPN-09 (6) and OPC-14 (3) respectively (Table 1). A total number of 55 amplified fragments was scored across eight varieties of ginger for the selected primers, and was used to estimate genetic relationships among themselves. Out of 55 fragment obtained, only 25 fragments (45.5%) were polymorphic. The pattern of RAPD produced by the primers OPA-19, OPN-10 and OPN-09 are shown in Figure 1. The profile generated by using OPN-10 are polymorphic. The genetic variation through RAPD markers has been highlighted in a number of medicinal plants (Bai et al. 1997, Rout et al. 1998, Pal

Table 1. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected random decamers.

Name of primer	Sequence of the primer	Total number amplified products	Number of polymorphic products	Size range [kb]
OPA-02	5′-TGCCGAGCTG-3′	5	2	0.5–1.2
OPA-13	5′-CAGCACCCAC-3′	4	2	0.6–1.7
OPA-19	5′-CAAACGTCGG-3′	4	3	0.8–2.4
OPA-20	5′-GTTGCGATCC-3′	4	2	1.3–2.1
OPN-09	5′-TGCCGGCTTG-3′	6	3	0.5–1.6
OPN-10	5′-ACAACTGGGG-3′	6	3	0.7–1.5
OPN-15	5′-CAGCGACTGT-3′	6	3	0.8–2.0
OPN-20	5′-GGTGCTCCGT-3′	5	2	0.6–1.7
OPC-04	5′-CCGCATCTAC-3′	4	1	0.5–1.1
OPC-09	5′-CTCACCGTCC-3′	4	2	0.7–1.2
OPC-14	5′-TGCGTGCTTG-3′	3	1	0.5–1.6
OPC-18	5′-TGAGTGGGTG-3′	4	1	0.8–2.0

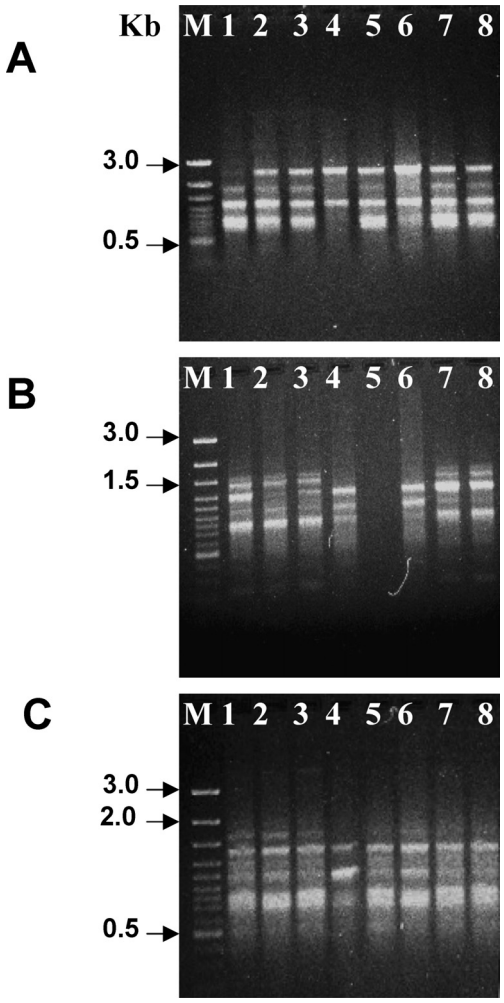

Figure 1. RAPD patterns of eight varieties of ginger generated by the primer OPA 19 (A), OPN 10 (B) and OPN 09 (C). M–Molecular weight ladder (Kb), 1–8 reflect the different varieties of ginger.

and Raychaudhuri 2003, Rout 2006). The present results show the narrow variation within some of the varieties. The similarity matrix was obtained after multivariant analysis using Nei and Li's coefficient and is presented in Table 2. The matrix value was ranged from 0.34 to

Table 2. Similarity matrix of eight varieties of ginger.

	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8
Z1	1.0							
Z2	0.60	1.0						
Z3	0.65	0.68	1.0					
Z4	0.34	0.46	0.48	1.0				
Z5	0.54	0.59	0.65	0.43	1.0			
Z6	0.43	0.65	0.60	0.48	0.54	1.0		
Z7	0.60	0.68	0.72	0.46	0.68	0.68	1.0	
Z8	0.54	0.80	0.68	0.44	0.60	0.65	0.74	1.0

Z1, Surabhi; Z2, Jugijan; Z3, Nadia; Z4, S-558; Z5, Gurubathan; Z6, Nahfrey; Z7, ZO-17; Z8, Turia local.

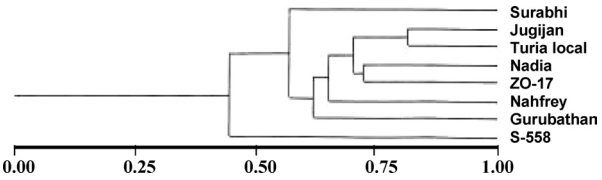

Figure 2. Dendrogram of eight verities of ginger generated by UPGMA based on RAPD markers.

0.74, with a mean value of 0.54. The high matrix values indicated that there varieties were distantly related to each other. Nayak et al (2005) reported that there was high genetic variation among the 16 varieties of ginger. The high difference in gene diversity among varieties reveals the presence of strong genetic structure between them and thus siginificant differences exist in the genotypic diversity among themselves. The similarity matrix obtained in the present study was used to construct a dendrogram with the unweighted UPGMA method and resulted in their distant clustering in the dendrogram (Figure 2). The dendrogram shows two major clusters. The first major cluster had only one variety 'S-558' with 43% similarity with other seven varieties. Second major cluster having seven varieties and again divided into two minor clusters. One minor cluster had six varieties ('Jugijan', 'Turia local', 'Nadia', 'Zo-17', 'Nahfrey' and 'Gurubathan') and other having only one variety 'Surabhi'. The second minor cluster

further divided into two sub-minor clusters. One sub-minor cluster had only one variety 'Gorubathane'. Another sub-minor cluster having five varieties; 'Nadia' and 'ZO-17' had 78% similarity among themselves and 70% similarity with 'Jugijan' and 'Turia local'. 'Jugijan' and 'Turia local' were having 81% similarity among themselves. 'Nahfrey' had 64% similarity with 'Jugijan', 'Turia local', 'Nadia' and 'ZO-17'. The differences in number of individuals estimated by RAPD markers in this study are similar to the result obtained by Rajaseger et al. (1997) in RAPD studies of the *Ixora coccinea* and *I. javanica*. They also found that the taxa-specific RAPD bands could be utilized to define the identification.

The present findings include the identification and genetic variation within eight varieties of ginger. The dendrogram shows the distant variation within the varieties. The genetic relation through RAPD markers provides a reliable method for identification of varieties than morphological characters. This investigation as an understanding of the level and partitioning of genetic variation within the varieties would provide an important input into determining efficient management strategies. The genetic variability in a gene pool is normally considered as being the major resource available to breeders for ginger improvement program.

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