Genetic Diversity Analysis of Ginger (*Zingiber officinale* Roscoe.) Genotypes Using RAPD Markers

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Abstract: Ginger (*Zingiber officinale* Roscoe.) is an important spice crop in Bangladesh as well as in all over the world for its economical and medicinal values. A measure of the existing genetic diversity is essential for planning a meaningful breeding strategy. Moreover, assessment and characterization of genetic diversity of the available germplasm is important to know the source of gene for particular trait of interest. The present investigation was undertaken to assess the genetic diversity among eight ginger genotypes using RAPD markers. A total of 16 distinct DNA fragments ranging from 100–1000 bp were amplified by using three selected primers of which 10 (62.50%) were polymorphic. BARI ada-1 was more homogenous than others and Syedpuri was found less homogenous showing the low intra-variety similarity value (75.56). the genotype 'Syedpuri' was found as more diversified from the viewpoint of lowest intra-variety similarity index value, highest gene diversity, proportion of polymorphic loci and highest level of genetic variation. The cluster analysis indicated that the eight genotypes were grouped into two major clusters. 'Indian' alone formed the first major cluster while the second major cluster had seven genotypes and was divided into two minor clusters. China and Sherpuri genotype pair was very close to each other with the lowest genetic distance (0.03). On the other hand, Indian and Syedpuri pair was more distant to each other with the highest genetic distance (0.55). RAPD analysis revealed a considerable level of polymorphism among the studied genotypes. The genetic variation thus detected has significance for ginger improvement programs.
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1. Introduction

Ginger (*Zingiber officinale* Rose.), a herb with underground tuberous aromatic stems (rhizomes) is a member of Zingiberaceae family. It is a plant of pungent and spicy aroma with great economic and medicinal value. It is an important spice crop of Bangladesh. The major ginger producing countries of the world are India, Pakistan, Nepal, China, Japan, Taiwan, Malaysia, Indonesia, Jamaica, Sierra Leone, Nigeria, Mauritius, and Australia. In Bangladesh, ginger grows well in Rangpur, Nilphamari, Tangail, Rangamati, Bandarban, Khagrachari and Chittagong district (Choudhury *et al.*, 1998). A number of food products like ginger bread, confectionery, ginger-ale, dried ginger powders, certain curried meats, table sauces, pickles and soft drinks are prepared from ginger. It is also reported to be used in many herbal and veterinary medicines.

Crop genetic resources with a broad genetic base and high variability are vital to crop improvement program. Assessment and characterization of the state of the existing genetic diversity within the taxon is critical for planning a meaningful breeding strategy (Cooper *et al.* 2001). A number of techniques are available for studying the variability of crop germplasm like morphological traits, total seed protein, isozymes and various types of molecular markers. However, molecular markers provide powerful and reliable tools for assessing variations within crop germplasm and for studying evolutionary relationships (Gepts, 1993). Among them, RAPD markers, generated by the polymerase chain reaction (PCR) has widely been using to assess genetic variation at gene level (Welsh and McClelland, 1990; William *et al.,* 1990). The technique of RAPD gained importance due to its simplicity, efficiency and non-requirement of sequence information (Karp *et al.,* 1997). The intention of this investigation was to assess genetic diversity and relatedness of the ginger genotypes by PCR based RAPD technique as it is very much important for further improvement of ginger.

2. Material and Methods

Eight ginger genotypes collected from different regions of Bangladesh were used in this study. Genomic DNA was extracted from fresh leaves by using the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). The DNA samples

were evaluated both quantitatively and qualitatively by 1% agarose gel electrophoresis and genomic DNA concentration was estimated by spectrophotometer at a wavelength of 260 nm using the Spectronic® GenesisTM (Spectronic Instruments Inc., USA). Before PCR, the final DNA concentration of each sample was adjusted to 25 ng/µl.

Twelve primers, corresponding to kits A, B, C, D and E from Operon Technologies, (Alameda, California, USA) were initially tested and finally three primers (OPA-05, OPC-01 and OPE-02) exhibiting higher quality bands with minimal smearing and good resolution was selected for DNA amplification adopting the procedure of William *et al.* with some modifications. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing, and potential for population discrimination. Finally, three primers were selected for the analysis of the whole sample of the eight genotypes.

PCR reactions were performed for each DNA sample with single primer in a 10 µl reaction mix containing 1 µl *Taq* DNA polymerase buffer (10X), 2.5 µl primer (10 µM), 1 µl dNTPs (250 µM each), 1 unit *Taq* DNA polymerase (0.3 µl) (Genei Pvt. Ltd., Bangalore, India) and 100 ng (4 µl) of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf, Germany) programmed for a initial denaturation at 94°C for 2 min, followed by 40 cycles of 20 s denaturation at 94°C, 30 s annealing at 38°C and 1 min extension at 72°C. After the last cycle, a final step of 10 min at 72^0C was added to allow complete extension of all amplified fragments. Reactions were held at 4^0C after completion of cycling program. Tubes containing all reaction components except DNA were used as negative control. Amplicons were analyzed electrophoretically on 1.5% agarose gel (Fisher Biotech, USA) containing ethidium bromide in 1X TBE buffer at 120 V for 1 hr. A molecular weight marker DNA (100 bp ladder) was also electrophoresed alongside the PCR products. The stained gels were documented using UV Transilluminator (UVP Ltd., Trinity hall estate, Cambridge, UK).

Distinct RAPD bands were given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0) separately for each individual and each primer. The scores were then pooled to construct a single data matrix. This was used for estimating polymorphic loci, Nei's (1973) gene diversity, Shannon's Information index (Lewontin 1972) and the UPGMA dendrogram of the populations based on Nei's (1972) genetic distances using the POPGENE (Version 1.31) (Yeh *e. al.*, 1999) software package. The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula:

Similarity index (SI) = $2 N_{xy}/(N_x + N_y)$

Where N_x = the total number of fragments detected in individual 'x'; N_y = the total number of fragments shown by individual 'y' and Nxy = the number of fragments shared by individuals 'x' and 'y' (Lynch 1990). Between population similarity (S_{ij}) was calculated as the average similarity between randomly paired individuals from populations i and j (Lynch 1991).

3. Results and Discussion

RAPD analysis of the studied genotypes detected a conspicuous level of polymorphism among them. Fig. 1(a-c) shows the RAPD profile of eight ginger genotypes using primer OPA-05 (Fig. 1a), OPC-01(Fig. 1b) and OPE-02(Fig. 1c). Three out of twelve primers, (OPA-05, OPC-01 and OPE-02) generated a total of 16 bands of which 10 (62.50%) were polymorphic.

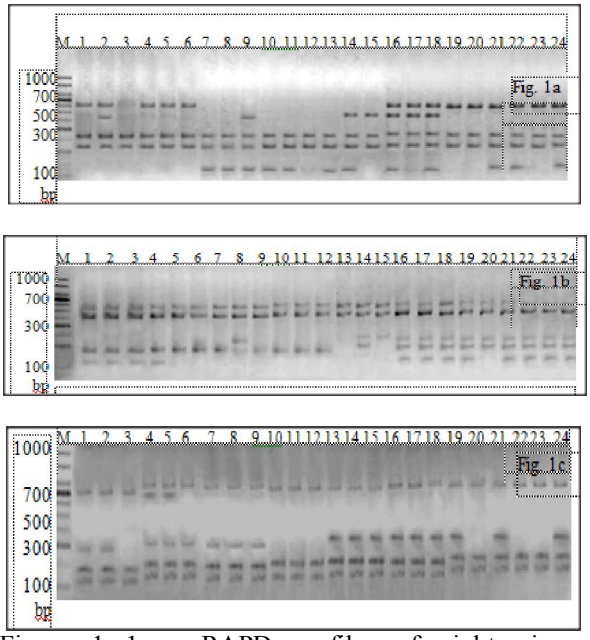

Figure 1a-1c. RAPD profiles of eight ginger genotypes using primer OPA-05 (Fig. 1a), OPC-01(Fig. 1b) and OPE-02(Fig. 1c). (M: Molecular weight marker (100 bp DNA ladder), Lane 1-3: Indian, Lane 4-6: Wild, Lane 7-9: Chittagangi, Lane 10-12: Fulbaria, Lane 13-15: Syedpuri, Lane 16-18: BARI ada-1, Lane 19-21: China and Lane 22-24: Sherpuri)

The primer OPC-01 produced maximum number of bands (6) and the other two primers OPA-05 and OPE-02 generated 5 bands each. The highest number of polymorphic bands (4) was produced by primer OPC-01 and thus it showed a higher level of polymorphism. The other two primers OPA-05 and OPE-02 generated 3 polymorphic bands of each. On an average, the three primers yielded 5.33 scorable bands per primer and 3.33 polymorphic bands per primer (Table 1).

Table 1. RAPD primers with corresponding bands scored and their size range together with polymorphic bands observed in eight ginger genotypes (three replications for each genotype).

Primer Codes	Sequences (5´-3´)	Total number of bands scored	Size range (bp)	Number of polymorphic bands	Polymorphic loci (%)
OPA-05	TGCCGAGCTG	5	100-1000	3	62.50
OPC-01	GTGAGGCGTC	6	100-1000	4	
OPE-02	GTTTCGCTCC	5	100-1000	3	
Total		16		10	
Average		5.33		3.33	

The highest intra-variety similarity indices (Si) value was found in BARI ada-1 (95.24%) followed by that of Chittagongi, Sherpuri, Wild, Fulbaria, China, and Indian, Syedpuri, respectively (Table 2). The individuals of BARI ada-1 were more homogenous having the highest intra-variety similarity indices (S_i) value (95.24%). Greater extent of genetic variation was found between the individuals of Syedpuri having the lowest intra-variety similarity indices (S_i) value (75.56%). The inter-variety similarity indices (Sij) between BARI ada-1 and Sherpuri were found to be the highest (92.76%) indicating the genetic distance between the two genotype was low and the lowest band sharing value (58.36%) were observed between Indian and Syedpuri indicating existence of greater genetic distance between these populations (Table 2). The values for intra-variety similarity indices (Si) were higher (average 88.44%) than intervariety similarity indices (Sij) (average: 79.02%) indicating less genetic variation between two individuals of same genotype than that of different genotypes.

The number and proportion of polymorphic loci was found to be highest, 4 and 25%, respectively in Syedpuri. Indian, Wild, Chittagongi, Fulbaria and Sherpuri each had 2 (12.50%) polymorphic loci. Syedpuri was likely to be the most diversified genotype with the highest gene diversity value (0.1102) and Shannon's information index (0.1575). On the other hand, BARI ada-1 was found to be the least diversified among the eight genotypes having the lowest value of gene diversity, Shannon's information index and proportion of polymorphic loci (%).

Table 2. Estimates of genetic variation, number and proportion polymorphic loci, gene diversity and Shannon's Information index obtained in eight ginger genotypes.

Genotype	No. of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity (h)	Shannon's Information index (I)
Indian	2	12.50	0.0492	0.0724
Wild	2	12.50	0.0492	0.0724
Chittagongi	2	12.50	0.0375	0.0596
Fulbaria	2	12.50	0.0375	0.0596
Syedpuri	4	25.00	0.1102	0.1575
BARI ada-1	1	6.25	0.0305	0.0426
China	3	18.75	0.0797	0.1149
Sherpuri	2	12.50	0.0492	0.0724

The Nei's (1972) genetic distance (D) of different genotype pairs are shown in Table-3. Comparatively higher genetic distance was observed between Indian vs. Syedpuri (0.5531), Chittagongi vs. BARI ada-1 (0.4567) and Indian vs. Chittagongi genotype pair than other genotype combinations. The lowest genetic distance (0.0302) was found in China vs. Sherpuri genotypic pair. The wide range of genetic distance (0.0302 to 0.5531) indicated that the genotypes were genetically different from each other (Table 3).

Table 3. Summary of Nei's (1972) genetic distance values between eight ginger genotypes

Genotype	1	2	3	4	5	6	7	8
1	***							
2	0.17	***						
3	0.43	0.16	***					
4	0.34	0.17	0.09	***				
5	**0.55**	0.36	0.24	0.15	***			
6	0.39	0.39	0.46	0.38	0.25	***		
7	0.29	0.18	0.28	0.17	0.21	0.12	***	
8	0.26	0.22	0.31	0.22	0.32	0.12	**0.03**	***

Here; 1, 2, 3, 4, 5, 6, 7 and 8 denotes, Indian, Wild, Chittagangi, Fulbaria, Syedpuri, BARI ada-1, China and Sherpuri genotypes of ginger

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the eight genotypes sof ginger into two main clusters. The first major cluster had only one genotype -Indian. Second major cluster had the rest seven genotypes and was divided into two minor clusters. One minor cluster had four genotypes (Wild, Chittagangi, Fulbaria and Syedpuri) and other having three genotypes BARI ada-1, China and Sherpuri. In first minor cluster Wild solely formed sub-minor cluster-I while Chittagangi, Fulbaria and Syedpuri formed sub-minor cluster-II. Sub minor cluster-II was again divided into two groups - Chittagangi was in group-I and Fulbaria and Syedpuri was in group II. On the other hand, in second minor cluster only BARI ada-1 formed group-I and China and Sherpuri were in group-II (Fig. 2).

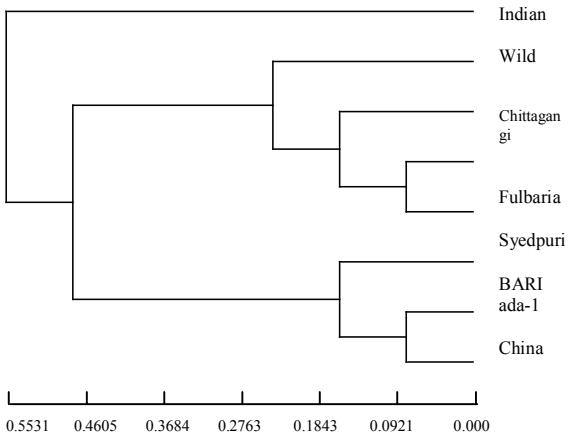

Figure 2. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between ginger genotypes according to RAPD analysis.

Being a poorly studied genome, little information is available on the molecular characterization of ginger. This is why, there is a need to conduct studies to evaluate the genetic diversity of ginger for breeding and conservation purposes. This investigation presented a sufficient amount of variation among the studied genotypes indicated that the RAPD method could be used as an effective tool for molecular genetic analysis of various genotypes of ginger. However, large number of samples from all the AEZ of Bangladesh and higher number of primers would be useful to draw a more definite conclusion. Through this study, we have assessed, for the first time the genetic relationship among some ginger genotypes of Bangladesh. The result of the present study can be used as a guideline for future diversity assessment and genetic analysis of ginger genotypes.

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