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# Molecular Diversity Analysis of Some Local Ginger (*Zingiber officinale*) Genotypes Using RAPD Markers

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***Abstract*—***Ginger (Zingiber officinale) rhizomes have been widely used as a spice and flavoring agent in foods and beverages in Bangladesh as well as in all over the world for its economical and medicinal values. The present investigation was undertaken for the assessment of 13 local ginger genotypes collected from different region of Bangladesh through 7 RAPD primers. Genomic DNA was extracted from ginger genotypes using CTAB method. A total of 34 distinct and differential amplification bands ranging from 150-1200 bp were observed with an average of 1.14 polymorphic bands per primer. The overall gene diversity was detected 0.8052 and the value of PIC was detected 0.7532. The RAPD marker generate enough polymorphism for possible use in diversity studies through cluster analysis and principal component analysis (PCA). PCA classified 13 ginger genotypes into four groups and showed in two dimensional scatter plot. The genetic similarity coefficients among genotypes ranged from 0.103 to 0.654. Cluster analysis based on Jaccard's similarity-coefficient using UPGMA grouped the genotypes into two clusters: Cluster A and Cluster B. The cluster 'A' had only one genotype Kaptai local and the second cluster 'B' had rest of twelve genotypes. The prevalence level of polymorphism in the local genotypes of ginger will help to breeders for ginger improvement program.*

***Keywords*— *Ginger, Molecular diversity, Polymorphism, RAPD.***

## I. INTRODUCTION

Ginger (*Zingiber officinale*) is a herbaceous perennial vegetatively propagated underground tuberous aromatic stems (rhizomes). It is a source of pungent and spicy aroma with great economic and herbal medicinal value. Ginger is rich in secondary metabolites oleoresin and volatile oil (Barnes *et al*., 2002; Sweetman, 2007) and also having more than 400 different bio chemicals which has the medicinal and spicy value (Garner-Wizard *et al*., 2006). It is an important spice as well as cash 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 (Jansen, 1981; Yiljep *et al*., 2005). In Bangladesh, ginger grows well in Rangpur, Nilphamari, Tangail, Rangamati, Bandarban, Khagrachari and Chittagong district (Choudhury *et al*., 1998). The food, perfumery, and pharmacy industries are the main outlets where ginger is used. It is found to be used in various pickles, cake and chatni and also used in preparing medicine like ayuratic, homeopathic, and also allopathic.

The average yield of ginger is about 15-25 tons/ha which is very low compared to world statistics (BBS 2017). The use of local rhizome, traditional varieties and many biotic and abiotic stress are the major constrains of low yield of ginger. Development of high yielding varieties is one of the challenges for the breeders. Crop genetic resources with a broad genetic base and high variability are vital to crop improvement program. Most of the crop improvement programs of ginger are restricted to the assessment and selection of naturally occurring clonal variations (Rout *et al*., 1998; Palai and Rout,2007). Assessment and characterization of the existing genetic diversity within the taxon is critical for planning a meaningful breeding strategy (Cooper *et al*., 2001).

A number of techniques such as morphological traits, total seed protein, isozymes and various types of molecular markers are available for studying the variability of crop germplasm. However, molecular markers provide powerful and reliable tools for assessing variations within crop germplasm and for studying evolutionary relationships (Gepts,1993). Molecular markers are not prone to environmental influences and accurately characterize the plants portraying the extent of

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genetic diversity among taxa (Bennett and Smith, 1991; Rodriguez *et al*., 1999; Das *et al*., 2001). Among different molecular markers RAPD techniques 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 achieved importance due to its simplicity, efficiency and non-requirement of sequence information (Karp *et al*., 1997). RAPD markers have been widely used for identification of genetic relationship among cultivars (Tosti and Nejri,2002). Therefore, the present investigation was carried out to study polymorphism among the different local ginger genotypes through RAPD marker and diversity analysis at DNA level for ginger improvement programs.

## II. MATERIALS AND METHODS

The experiments were carried out at Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh. Thirteen local ginger genotypes were collected from different area of Bangladesh and used as experimental materials. The ginger varieties were Kurigram local, Tangail local, Jamalpur local, Modupur local, B. Baria local, Rangamati local, Thakurgoan local-1, Thakurgoan local-2, Lalmonirhat local-1, Lalmonirhat local-2, Lalmonirhat local-3, Rangpur local and Kaptai local.

### 2.1 Seedling raising

Good quality, disease free and healthy rhizome were sown in plastic pots. All management practices were done for raising quality seedlings from those materials. Fresh and tender leaves were collected at young leaf stage of plant for extraction of DNA.

### 2.2 Extraction and quantification of DNA

Total genomic DNA was extracted from each ginger genotypes by cetyltrimethyl ammonium bromide (CTAB) method with slight modification according to Frey *et al*., (2004). The extracted DNA was purified by iso-propanol and treated with 10µg/ml RNase for 20-25 min at 37°C to remove the RNA. The purified DNA was dissolved in TE buffer and quantification of DNA was done through electrophoresis on 1% agarose gel staining by ethidium bromide. Finally sample DNA was stored at -20°C freezer for further use.

### 2.3 Primer selection and PCR amplification

Seven RAPD primers were selected for this study PCR reaction were performed in a 10µl reaction mix containing 5.0 µl 2X Taq master mix, 1.0 µl primer, 2.0 µl sterile deionized water, 2.0 µl (20-25 ng) genomic DNA. Samples were subjected to the following thermal profile for amplification in a thermocycler: 4 min at 95°C for initial denaturation followed by 31 cycles of 45 sec denaturation at 94°C, 30 sec at 35°C (annealing) and 1 min at 72°C (extension) and a final extension step at 72°C for 5 min. Electrophoresis was carried out in 1X TBE buffer on a 2% agarose gel and amplified fragments were visualized by staining with ethidium bromide. The amplified bands were visually scored as present (1) and absent (0) separately for each individual primer. The scores of bands were pooled to create a single data matrix to estimate polymorphic loci, polymorphic information content, principal component analysis and population structure analysis. Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram was constructed using a computer programme, POPGENE (Version 1.31) based on Nei's (1972) genetic distance used to determine similarity matrix following Dice coefficient with SAHN subprogram (Yeh*et al*., 1999).

## III. RESULT AND DISCUSSION

Seven decamer RAPD primers *viz*. OPG19, OPJ13, OPM07, OPM05, OPP13, OPW03, and OPX04 showed reproducible and distinct polymorphic amplified bands (Fig. 1-7). A total of 34 bands were scored of which 8 (23.50%) were polymorphic. The size of the amplified products ranged from 150-1200 bp (Table-1). The highest (3) number of polymorphic band was produced by the primer OPP 13. The primer OPM 05 and OPW 03 were not able to regenerate any polymorphic band. Maximum 50% of polymorphism was recorded in the primerOPP 13 and OPM 07followed by primer OPJ13 (28.57%), OPX04 (20%) and OPG 19 (16.67%).

The frequency of the major allele ranged between 0.2308 to 0.4615 with an average value of 0.3187 (Table 2). Polymorphic Information Content (PIC) value for the 7 markers ranged from 0.6560 to 0. 8496 and the average PIC value was 0.7532. The highest PIC value (0.8496) was obtained in the primer OPJ 13 and the lowest PIC value 0.6560 was obtained for OPW 03 and OPM 05. PIC value revealed that, OPP 13 and OPJ 13 were considered as the best marker for 13 ginger genotypes. Gene diversity ranged between 0.6982 (OPW 03) to 0.8496 (OPP 13 and OPJ 13) with an average of 0.8023. The results indicated that, the 13 local ginger genotypes present a degree of homozygosity and also considerable intra-varietal group diversity and a certain degree of genetic differentiation and polymorphism. Mia *et al*. (2014) investigated on 8 Bangladeshi ginger genotypes whereas the highest genetic distance (0.5531) was observed between Indian vs. Syedpuri and the lowest genetic identity (0.0302) was found in China vs. Sherpuri genotypic pair of ginger.

Output of the population structure analysis was collected using the STRUCTURE harvester (Earl D. A., 2012) to identify 2 as the best K value based on the LnP (D) and Evanno's ΔK (Evanno G. *et al*., 2005). The value of K= 2 means, the total population divided into two sub groups presented in Fig. 8. Bayesian analysis using

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STRUCTURE characterized the population structure for tested local ginger germplasm. Here only green and only red were pure variety. Both color indicated admixture of character. Kurigram local, Tangail local, Thakurgoan local-2 (red color) and Rangamati local, Thakurgoan local-1, Lalmonirhat local-2, Lalmonirhat local-3, Rangpur local, Kaptai local (green color) showed pure character.

Principal components analysis (PCA) analysis was conducted also using the PAST software. All 13 ginger genotypes were classified into four groups and showed in two dimensional scatter plot. All the groups were separated from each other (Fig. 9). Highest distance was showed between Group I and Group III. Group I represents Kaptai local and Group III represents Thakurgoan local-2. It indicated that, material selection from Group I and Group III will be more effective for ginger improvement program.

The value of pair-wise comparisons Nei's (1972) genetic distance between 13 ginger genotypes were computed from combined data through 7 primers, ranging from 0.103 to 0.654. The highest genetic distance 0.654 was observed in Kaptai local vs. Kurigram local varietal pair whereas lowest value was observed in B. Baria local and Modhupur local varietal pair (Table 3).

**3.1 Cluster analysis**

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 13 genotypes of ginger into two main clusters: A and B. The first major cluster 'A' had only one genotype (Kaptai local) and the second major cluster 'B' had rest of twelve genotypes. The second major cluster was subdivided into two minor clusters (C & D) in which one cluster (D) was subdivided into two minor cluster which included Kurigram local, Tangail local and Jamalpur Local. The other cluster (C) had rest of the nine. This minor cluster was also subdivided into two clusters (E & F). In which cluster (E) is divided into two sub clusters (G and H). Modhpur Local, B. Baria Local, Rangamati Local and Thakurgoan Local-1 were grouped in Cluster (G) and Thakurgoan Local-2 formed in cluster (H). Cluster (I) and (J) were the subdivision of cluster (F). Lalmonirhat Local-1, Lalmonirhat Local-2 and Lalmonirhat Local-3 grouped in cluster (I) and Rangpur Local formed in cluster (J) (Fig. 10).

## IV. CONCLUSION

The result indicates that the low level genetic distance exists between varieties with their same or different origins. Kaptai local vs. Kurigram local showed highest Nei's genetic distance (0.654) as they are exit from different parental origin. On the other hand B.Baria local vs. Modhupur local varietal pair showed lowest genetic distance (0.103) as they are cultivated from same parental origin. This variation can be created by geographical origin. The result also reveals that the genetic base among these ginger genotypes is rather narrow. RAPD markers provide a fast, efficient technique for variability assessment that complements methods currently being used in genetic resource management. Being a poorly studied genome, little information is available on the molecular characterization of ginger. The result of the present study can be used as a guideline for future diversity assessment and genetic analysis of ginger genotypes.

## V. ACKNOWLEDGEMENT

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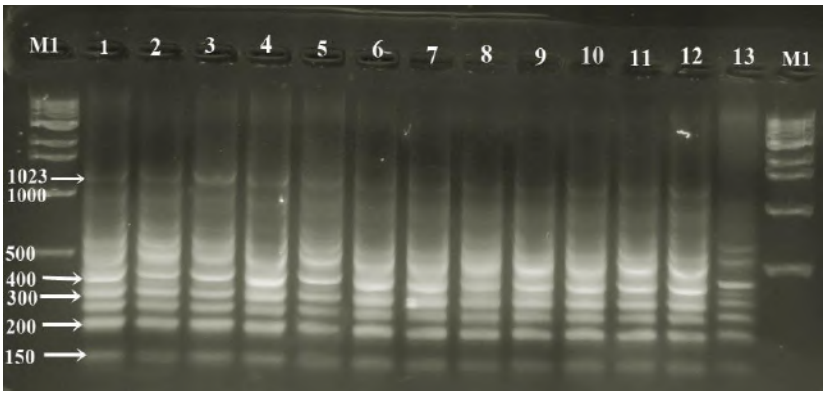


*Fig. 1: PCR amplification with RAPD primer OPG 19*

M1 & M2 = 1 kb DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local.

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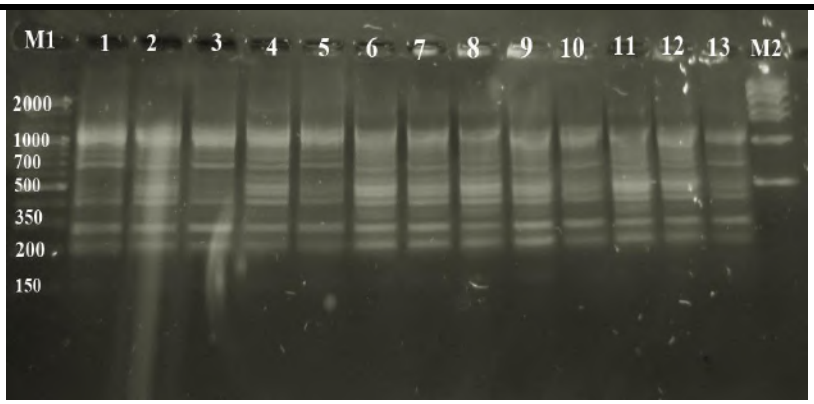


*Fig. 2: PCR amplification with RAPD primer OPJ 13*

M1= 100 bp & M2 = 1 kb DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local.

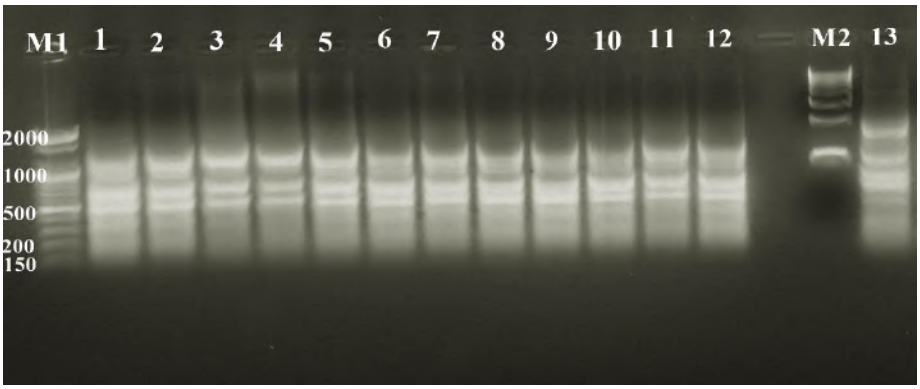


*Fig. 3: PCR amplification with RAPD primer OPM 05*

M1= 100 bp & M2 = 1 kb DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local.

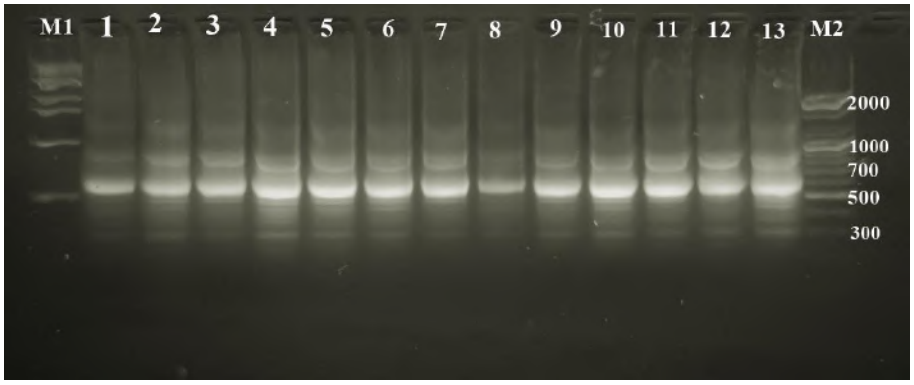


*Fig. 4: PCR amplification with RAPD primer OPM 07*

M1 & M2 = 100 bp DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local

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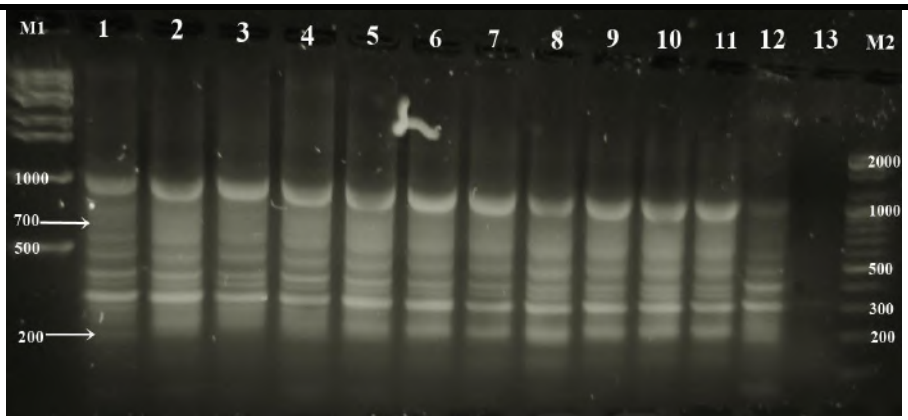


*Fig. 5:PCR amplification with RAPD primer OPP 13*

M1= 1 kb & M2 = 100 bp DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local

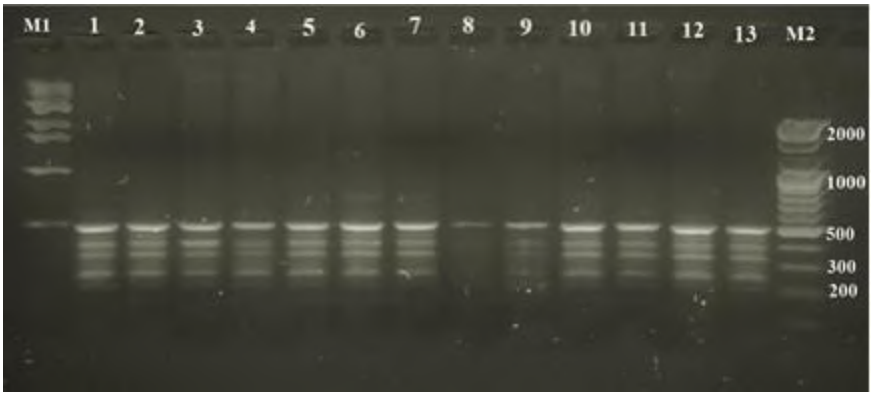


*Fig. 6: PCR amplification with RAPD primer OPW 03*

M1= 1 kb & M2 = 100 bp DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local

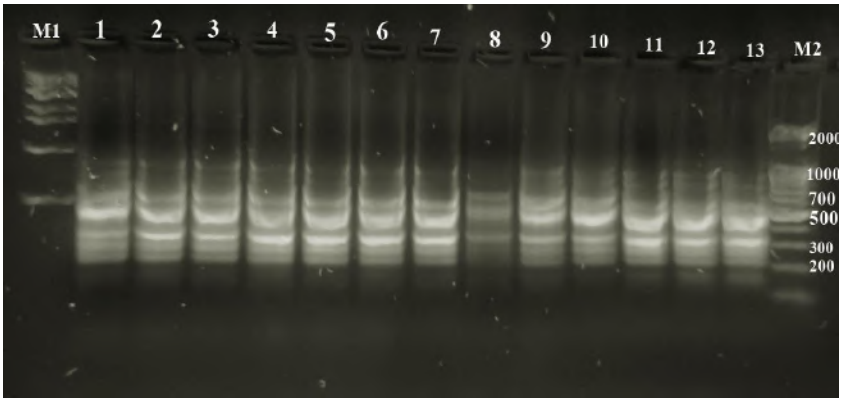


*Fig. 7: PCR amplification with RAPD primer OPX 04*

M1= 1 kb & M2 = 100 bp DNA ladder, Lane 1 = Kurigram local, Lane 2 = Tangail local, Lane 3 = Jamalpur local, Lane 4 = Modupur local, Lane 5 = B. Baria local, Lane 6 = Rangamati local, Lane 7 = Thakurgoan local-1, Lane 8 = Thakurgoan local-2, Lane 9 = Lalmonirhat local-1, Lane 10 = Lalmonirhat local-2, Lane 11 = Lalmonirhat local-3, Lane 12 = Rangpur local and Lane13 = Kaptai local.

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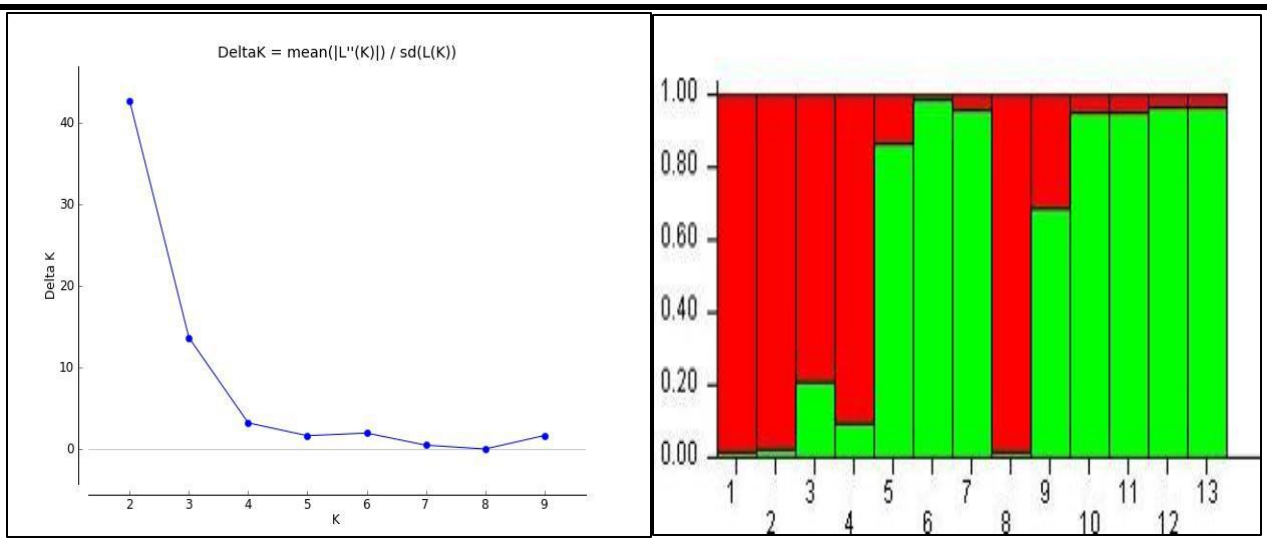


*Fig. 8: Population structure of 13 local ginger genotypes*

Population structure of 13 local ginger genotypes and 7 RAPD marker (K = 2) and graph of estimated membership fraction for K = 2. The maximum of adhoc measure ΔK determined by structure harvester was found to be K = 2, which indicated that the entire population can be grouped into two subgroups. Different colour within group indicates the proportion of shared ancestry with other group which has the same colour with the admixture.

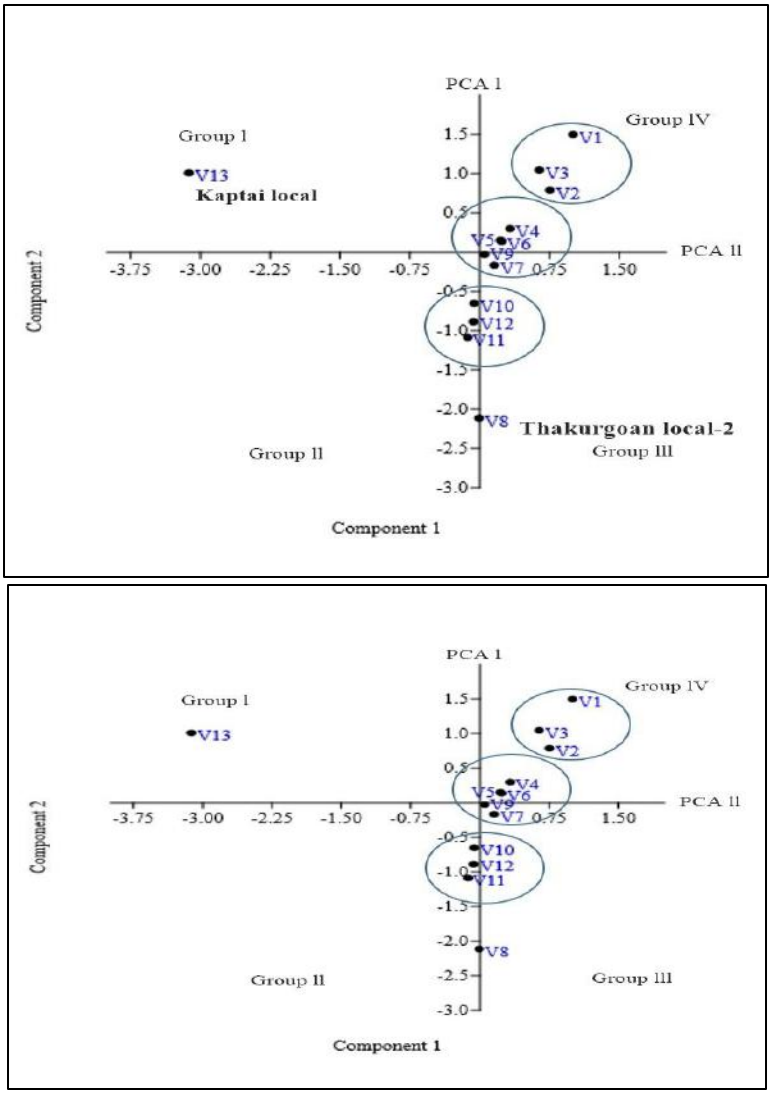


*Fig. 9: Principal Component Analysis of 13 ginger genotypes*

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PCA analysis of 13 local ginger genotypes based on 7 markers. PCA 1 and PCA ll represent first and second components, respectively.

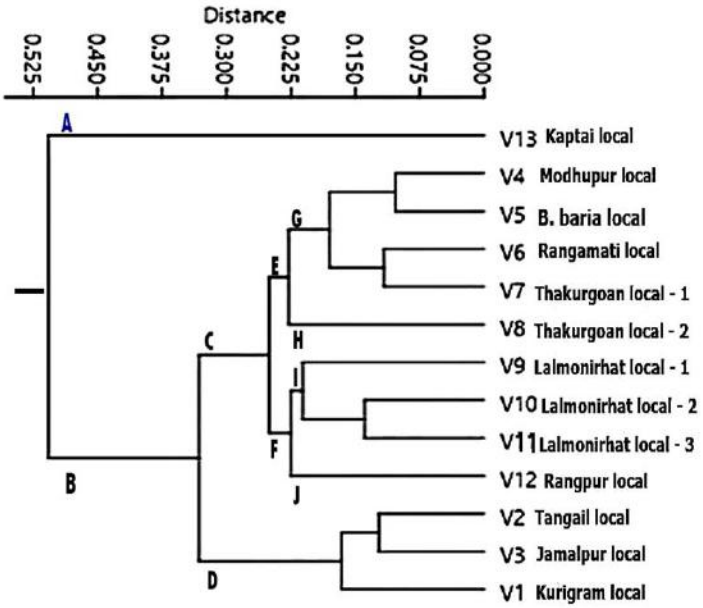


*Fig. 10: Figure Title: Cluster analysis*

Dendrogram of 13 ginger genotypes based n RAPD marker, according to the unweighted pair group mean algorithm (UPGMA) method based on a similarity matrix by PAST software

*Table.1: RAPD primers with corresponding band score and their size range together with number and percentage of polymorphic loci observed in 13 ginger genotypes*

| **Sl. No.** | **Primer** | **Primer Sequence ( 5´ to 3´ )** | **(G+C) %** | **Total no. of band scored** | **Number of polymer-phicbands** | **% of Polymo-rphism** | **Size ranges** |
|---|---|---|---|---|---|---|---|
| **1.** | OPG 19 | GTCAGGGCAA | 60 | 6 | 1 | 16.67 | **150- 1023** |
| **2.** | OPP 13 | GGAGTGCCTC | 70 | 6 | 3 | 50 | **200-1000** |
| **3.** | OPJ 13 | CCACACTACC | 60 | 7 | 2 | 28.57 | **225-1180** |
| **4.** | OPM 07 | CCGTGACTCA | 60 | 2 | 1 | 50 | **550-700** |
| **5.** | OPX 04 | CCGCTACCGA | 70 | 5 | 1 | 20 | **200-700** |
| **6..** | OPM 05 | AAGGGCGAGT | 60 | 3 | 0 | 0 | **500-1200** |
| **7.** | OPW 03 | GAGCGAGGCT | 70 | 4 | 0 | 0 | **242-500** |
| **Total** | | | - | 34 | 8 | | |
| **Mean** | | | **-** | **4.9** | **1.14** | **23.50** | |

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*Table.2: Major allelic frequency, gene diversity and PIC value of different ginger genotypes*

| Marker | Observations No. | Major Allele Frequency | Genetic Diversity | PIC Values |
|---|---|---|---|---|
| **OPG 19** | 13 | 0.3077 | 0.8402 | **0.7176** |
| **OPJ 13** | 13 | 0.4615 | 0.8439 | **0.8496** |
| **OPM 05** | 13 | 0.2308 | 0.8402 | **0.6560** |
| **OPM 07** | 13 | 0.2308 | 0.7574 | **0.8059** |
| **OPP 13** | 13 | 0.4615 | 0.8439 | **0.8245** |
| **OPW 03** | 13 | 0.2308 | 0.6982 | **0.6560** |
| **OPX 04** | 13 | 0.3077 | 0.7929 | **0.7630** |
| **Mean** | | **0.3187** | **0.8023** | **0.7532** |

*Table.3: Summary of Nei's genetic similarity and distance indices among the 13 ginger genotypes*

| | Kurigram local | Tangail local | Jamalpur local | Modupur local | B. Baria local | Rangamati local | Thakurgoan local-1 | Thakurgoan local-2 | Lalmonirhat local-1 | Lalmonirhat local-2 | Lalmonirhat local-3 | Rangpur local | Kaptai Local |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kurigram local** | 0.000 | | | | | | | | | | | | |
| **Tangail local** | 0.163 | 0.000 | | | | | | | | | | | |
| **Jamalpur local** | 0.169 | 0.123 | 0.000 | | | | | | | | | | |
| **Modupur local** | 0.299 | 0.229 | 0.191 | 0.000 | | | | | | | | | |
| **B. Baria local** | 0.283 | 0.225 | 0.183 | 0.103 | 0.000 | | | | | | | | |
| **Rangamati local** | 0.350 | 0.273 | 0.265 | 0.177 | 0.167 | 0.000 | | | | | | | |
| **Thakurgoan local-1** | 0.406 | 0.303 | 0.276 | 0.183 | 0.191 | 0.116 | 0.000 | | | | | | |
| **Thakurgoan local-2** | 0.411 | 0.348 | 0.303 | 0.221 | 0.243 | 0.237 | 0.210 | 0.000 | | | | | |
| **Lalmonirhat local-1** | 0.441 | 0.354 | 0.337 | 0.250 | 0.284 | 0.201 | 0.191 | 0.225 | 0.000 | | | | |
| **Lalmonirhat local-2** | 0.392 | 0.320 | 0.313 | 0.212 | 0.253 | 0.164 | 0.219 | 0.233 | 0.196 | 0.000 | | | |
| **Lalmonirhat local-3** | 0.402 | 0.380 | 0.356 | 0.250 | 0.254 | 0.206 | 0.258 | 0.226 | 0.227 | 0.139 | 0.000 | | |
| **Rangpur local** | 0.498 | 0.430 | 0.407 | 0.320 | 0.365 | 0.316 | 0.314 | 0.265 | 0.252 | 0.240 | 0.184 | 0.000 | |
| **Kaptai Local** | 0.654 | 0.630 | 0.606 | 0.505 | 0.520 | 0.513 | 0.553 | 0.466 | 0.483 | 0.434 | 0.383 | 0.340 | 0.000 |

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