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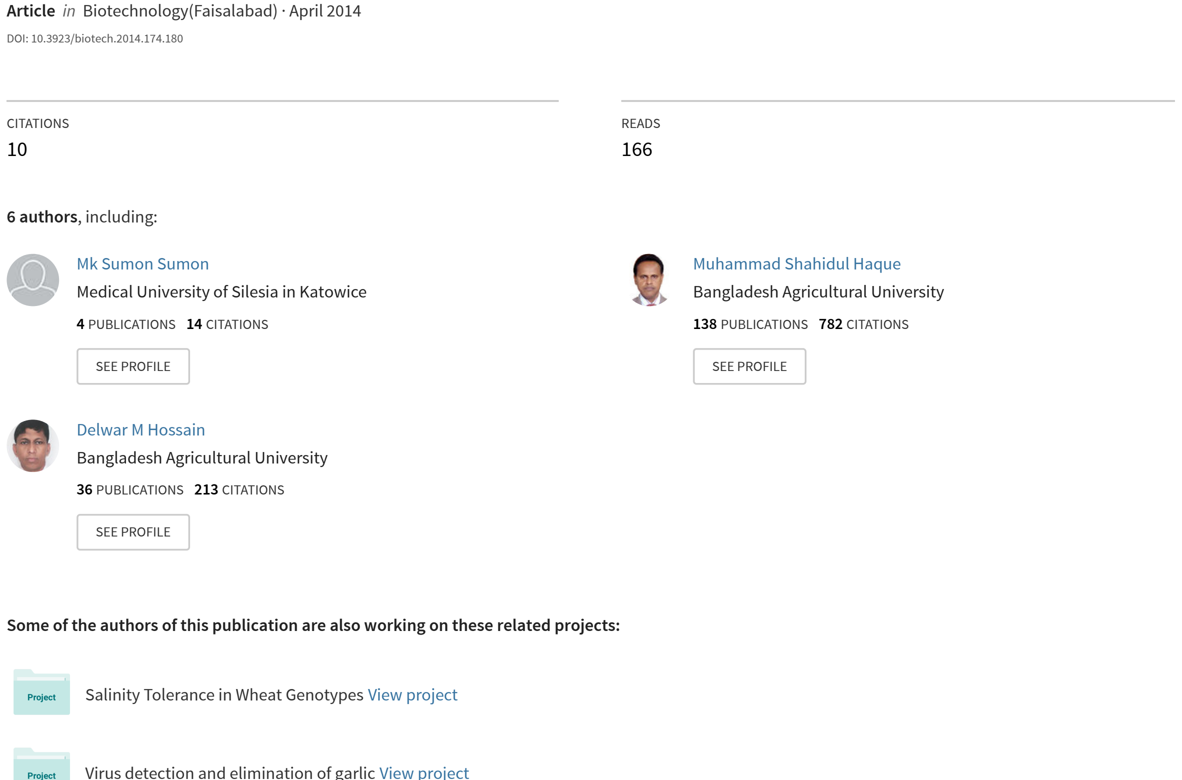
Article *in* Biotechnology(Faisalabad) · April 2014

DOI: 10.3923/biotech.2014.174.180



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ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 13 (4): 174-180, 2014
ISSN 1682-296X / DOI: 10.3923/biotech.2014.174.180
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DNA Fingerprinting and Genetic Diversity Analysis of Chilli Germplasm Using Microsatellite Markers

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Abstract: Microsatellite markers are useful tools for evaluating genetic diversity and DNA fingerprinting. The purpose of this study was to evaluate the genetic diversity within 22 chilli germplasm by using four microsatellite markers. All the microsatellite loci amplified by Polymerase Chain Reaction (PCR) were found polymorphic in all studied germplasm. A total of 27 alleles were detected and the number of alleles per marker ranged from 4-13. Based on Nei's genetic distance, the Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram grouped 22 chilli germplasm into 3 clusters; fifteen varieties Bogra morich BD-2043, Balijuri morich BD-2082, Kamranga morich, Tinn Tahori morich, Kalo morich, Angoor morich, Shada morich, Balujurii morich, BD-2011, BD-2035, BD-2005, Pepsicum morich, Dhani morich were grouped in cluster 1; Bindi morich, Altaf morich, Boro morich, BD-2025 were formed cluster 2 and Comilla morich, Sada gol morich and Ruma morich formed cluster 3. The values of pair-wise comparisons of Nei (1972) Genetic Distance (GD) between varieties were computed from combined data for the 4 primers and ranged from 0.704-0.926. The higher genetic distance indicated that these varieties were derived from different origin and could be utilized in breeding programme for traits of interest. From the difference between the highest and the lowest GD value, it was revealed that there were wide variabilities among 22 chilli varieties and genotypes. Higher genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. Thus microsatellite markers offer a potential, simple, rapid and reliable DNA fingerprinting method to evaluate genetic variation among the chilli germplasm. The findings of the present study have the potential applications in future breeding programme for the genetic improvement of chilli.

Key words: Chilli, SSR markers, DNA fingerprinting, genetic diversity

INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae. It is one of the most important spice crops in Bangladesh. It is cultivated in all parts of the country year around. The genus Capsicum has a wide genetic diversity composed by 27 species, being 5 domesticated and 22 semi-domesticated and wild ones (Reifschneider, 2000; Da Costa *et al*., 2006). Chilli fruits are the rich source of vitamin C (Ascorbic acid), A and vitamin E; apart from these, small quantity of protein, fats, carbohydrates and traces of minerals are also present and also a good source of chilli oleoresin which has varied uses in processed food, beverage industries and in pharmaceuticals. From the commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of plant lines. Genetic diversity is commonly measured by genetic distance or genetic similarity.

Bangladeshi chilli varieties have been developed traditionally by selection, hybridization and back crossing with locally adapted cultivars. The number of parental lines used in the breeding programmes is, however, study on genetic based is still in surface level. Genetic uniformity in crops can be undesirable in terms of vulnerability of the crop to epidemics and environmental disasters. An important source for the introduction of new traits is the existence of a genetically diverse pool of chilli germplasm available in the country but they are mostly lying unexplored. There is a strong need to collect this germplasm and their proper characterization and classification.

Availability of a large number of polymorphic markers enables precise classification of the cultivars and available germplasm. Several molecular markers viz., RFLP (Becker *et al*., 1995), RAPD (Tingey and del Tufo, 1993; Williams *et al*., 1990), SSRs (Levinson and Gutman, 1987),

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ISSRs (Blair *et al*., 1999), AFLP (Mackill *et al*., 1996; Zhu *et al*., 1998) and SNPs (Vieux *et al*., 2002) are presently available to assess the variability and diversity at molecular level (Joshi *et al*., 2000). With this view, the present study was made to evaluate the genetic diversity of 22 chilli genotypes using microsatellite markers.

MATERIALS AND METHODS

Young leaves were collected from 30 day old seedlings and DNA was extracted using PCI purification and ethanol precipitation method. For estimating concentration, DNA was quantified through spectrophotometer. The absorbance of each sample was measured at 260 nm and absorbance reading was revered. Using the absorbance readings, the original sample concentrations were determined according to the following equation:

$$\text{DNA conc. (ng } \mu\text{L}^{-1}) = \text{Absorbance} \times \frac{\text{Volume of distilled water } (\mu\text{L})}{\text{Amount of DNA sample } (\mu\text{L})} \times \text{C.F } (0.05) \times 1000$$

PCR analysis for microsatellite markers: Polymorphism survey of 22 chilli genotypes was carried out using thirteen microsatellite primers. Out of thirteen SSR primers, four primers (Hpms 1-41, Hpms 1-155, Hpms 2-2 and Hpms 2-21) showed clear polymorphisms which were used in genotyping the germplasms. The sequences of the primers are listed in Table 1.

The total volume of PCR cocktail for this study was 8.37 μL per sample. It contained 1 μL 10x PCR buffer, 1 μL dNTPs, 1 μL primer forward, 1 μL primer reverse, 0.25 μL *Taq* polymerase and 4.12 μL sterile dH_2O. Two microliter genomic DNA was added with 8.37 μL PCR cocktail. Template DNA was initially denatured at 94°C for 3 min followed by 34 cycles of PCR amplification with the following thermal profile; the 1 min of denaturation at 94°C, 1 min of primer annealing at 55°C and 2 min of primers extension at 72°C. Finally, 7 min incubation at 72°C was allowed for completion of primer extension. For checking amplification, the PCR products were electrophoretically resolved on 1.2% agarose gel in 0.5X TBE.

The level of polymorphisms among the 22 chilli germplasm was evaluated by calculating allele numbers and Polymorphic Information Content (PIC) values for each of the four SSR loci. The PIC or expected heterozygosity for each SSR marker was calculated based on the formula $Hn = 1-\Sigma p_i^2$, where p_i is the allele frequency for the i-th allele.

Polyacrylamide gel electrophoresis for microsatellite analysis: Before sample loading, both the gel and PCR products were preheated. During preheating of gel, the comb was again placed on the gel allowing sample to be loaded easily and 1200 mL 1x TBE buffer was added to the vertical chamber. The gel was pre-run for 30 min at 120 W to rise the temperature up to 50°C. Meanwhile, PCR-products and 5 μL 100 bp DNA ladder were preheated at 95°C for 5 min. After electrophoresis the gel was stained with Ethidium bromide. The individual bands in glass plate were stained and scored for analysis.

Data analysis: Only clear and unambiguous SSR markers were scored to the presence and absence of the corresponding band among the genotypes. Molecular weight for each amplified allele was measured in base pair using AlphaEase FC 4.0 software. Based on SSR markers data was subjected to cluster analysis using the software POPGENE (version 1.31). The allele frequency data from Power Marker Version 3.25 was used to export the data in binary format (Allele presence =1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2. The unweighted pair-group method with arithmetic means (UPGMA) dendrogram was drawn by using the software TREEVIEW. Nei (1972) genetic distance value was computed using the formula as described in the POPGENE (Version 1.31) software user manual.

RESULTS AND DISCUSSION

Genotypic performance of 22 chilli germplasms: Four microsatellite primers were employed to provide genetic diversity among 22 chilli germplasms. The primers showed polymorphism in 22 chilli germplasms. The microsatellite profiles of loci Hpms 1-41, Hpms 1-155, Hpms 2-2 and Hpms 2-21 are shown in Fig. 1-4.

Table 1: Sequences of microsatellite markers used for this study

Primer name	Size range (bp)	Sequence	Anneling temperature (°C)
Hpms 1-41	194-201	gggtatcatccgttgaaagttagg (Rev.) caagaggtatcacaacatgagagg (For.)	55
Hpms 1-155	197-226	acgaggcccaagctgttatgatgg (Rev.) ttgtcccgactctccattgacc (For.)	55
Hpms 2-2	153-160	gcaaggatgcttagttgggtgtc (Rev.) tcccaaaattaccttgcagcac (For.)	55
Hpms 2-21	300-315	tttttcaattgatgcatgacccgata (Rev.) catgtcattttgtcattgatttgg (For.)	50

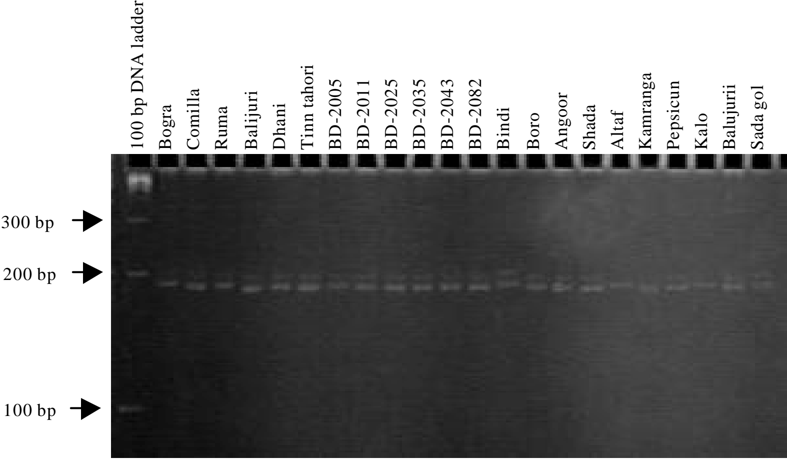

Fig. 1: Microsatellite profiles of 22 chilli germplasm at loci Hpms 1-41

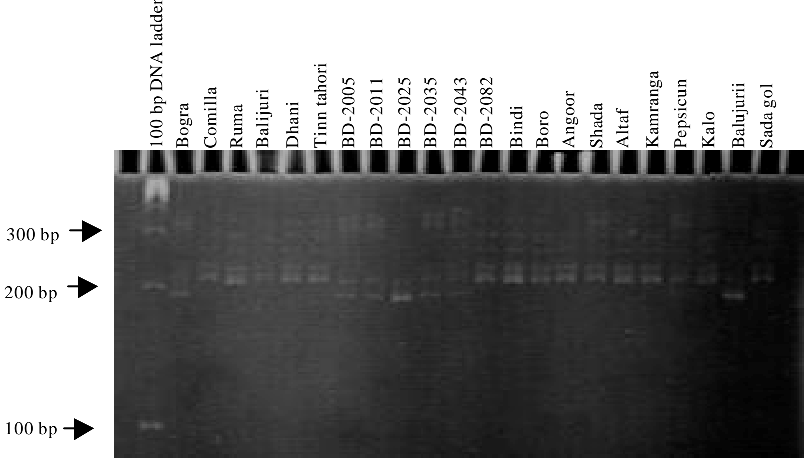

Fig. 2: Microsatellite profiles of 22 chilli germplasm at loci Hpms 1-155

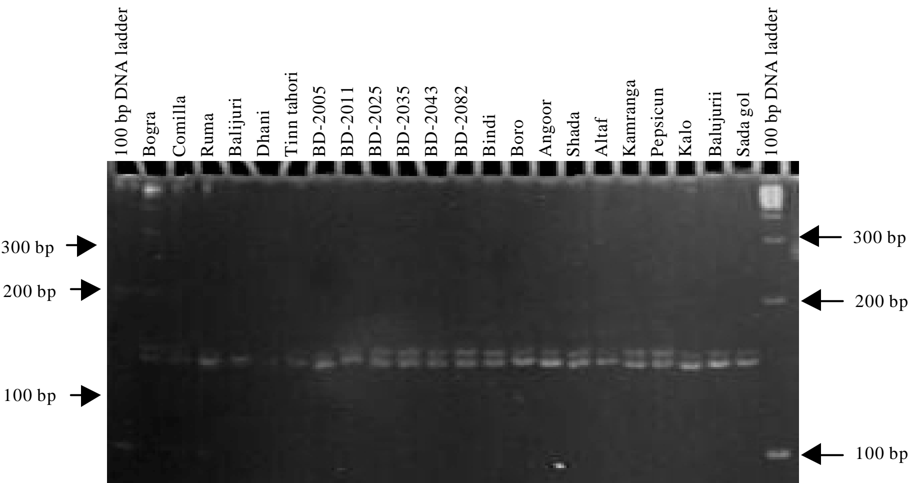

Fig. 3: Microsatellite profiles of 22 chilli germplasm at loci Hpms 2-2

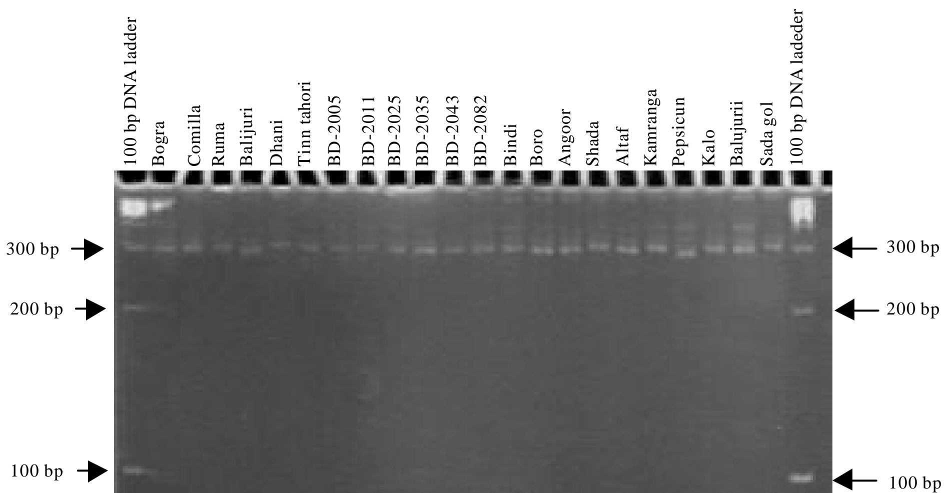

Fig. 4: Microsatellite profiles of 22 chilli germplasm at loci Hpms 2-21

Table 2: Microsatellite markers used the chromosome location of the loci, the number of alleles, gene diversity and the diversity index of 22 chilli germplasm

Primer name	Repeat motifs	Size range (bp)	No. of alleles	Rare allele	Gene diversity	Diversity index PIC = $1-\sum x_i^2$	Average PIC value
Hpms 1-41	(AT)6 (GT)32	194-201	4.00	0	0.7149	0.6664	
Hpms 1-155	(TA)3(GA)21	197-226	13.00	9	0.8554	0.8449	
Hpms 2-2	(GT)9	153-160	5.00	0	0.7810	0.7451	0.7236
Hpms 2-21	(AT)11(AC)9	300-315	5.00	1.00	0.6777	0.6381	
Mean	(ATAC)10		6.75	2.5	0.7572	0.7236	

The number of alleles ranged from 4-13 per locus (Table 2). The average number of alleles was 6.75. The study revealed that the primer Hpms 1-155 had the highest number of alleles (13) compared to primers Hpms 1-41, Hpms 2-2 and Hpms 2-21. As a measure of informativeness of microsatellite, the average PIC value was 0.7236 with the range of 0.6664 (Hpms 1-41), 0.8449 (Hpms 1-155), 0.7451 (Hpms 2-2) and 0.6381(Hpms 2-21). The highest PIC value was observed in Hpms 1-155 while lowest in Hpms 2-21. The size of the alleles for Hpms 1-41, Hpms 1-155, Hpms 2-2 and Hpms 2-21 of 22 chilli germplasms ranged from 194-201 bp, 197-226 bp 153-160 bp and 300-315 bp, respectively (Table 3).

Genetic distance: The mean of genetic distances between germplasms was used to evaluate the genetic diversity of different germplasms. The values of pair-wise comparisons of Nei (1972) Genetic Distance (GD) between genotypes were computed from combined data for the 4 primers, ranged from 0.704-0.926 (Table 4). Comparatively higher genetic distance (0.926) was observed between Angoor morich vs. Shada morich; BD-2082 vs. Kamranga morich; Angoor morich vs. Kamranga morich; Kamranga morich vs. Tinn Tahori morich; Balujurii morich vs. BD-2011; Boro morich vs. BD-2025 variety pairs than the other variety combinations. The lowest genetic distance (0.704) was found in different types of chilli germplasms. The average genetic distance among the 22 chilli germplasms was quantified as 0.764. The means of genetic distances between germplasms were used to evaluate the genetic diversity of different chilli germplasms.

From the difference between the highest and the lowest genetic distance value it was revealed that there were wide variability's among 22 chili varieties and genotypes. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that varieties those showed the highest genetic variation can be used as parental source for breeding line to improve chilli varieties.

Genetic similarity analysis using UPGMA: A dendrogram was constructed based on the Nei's genetic distance calculated from the 27 SSR alleles generated from the 22 chilli genotypes (Fig. 5). All 22 chilli germplasms could be easily distinguished. The Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 22 germplasms into three major clusters, fifteen varieties Bogra morich,

Table 3: Size and frequency of alleles at 4 SSR loci of 22 chilli genotypes

Locus/allele size (bp)	Allele frequency	Genotypes
Hpms 1-41		
194	0.2273	Balijuri, Tinn Tahori, Angoor, Shada, Kamranga
196	0.4091	Comilla, Dhani, BD-2005, BD-2025, BD-2035, BD-2043, BD-2082, Boro, Pepsicum
199	0.1818	BD-2011, Altaf, Balujurii
201	0.1818	Bogra, Ruma, Bindi, Kalo
Hpms 1-155		
197	0.0909	BD-2011, Balujurii
199	0.0455	BD-2025
200	0.0455	BD-2005
204	0.0909	Bogra, BD-2043
205	0.0455	BD-2035
213	0.0455	Kalo
214	0.0455	Ruma
216	0.0909	Bindi, Altaf
218	0.3182	Dhani, BD-2082, Boro, Angoor, Shada, Kamranga, Pepsicum
220	0.0455	Sada gol
222	0.0455	Comilla
224	0.0455	Tinn Tahori
226	0.0455	Balijuri
Hpms 2-2		
153	0.2273	Tinn Tahori, BD-2005, BD-2082, Kamranga, Kalo
155	0.1818	Ruma, BD-2035, Pepsicum
156	0.2727	BD-2025, Bindi, Boro, Angoor, Shada, Altaf
158	0.2273	Comilla, Balijuri, Dhani, BD-2043, Sada gol
160	0.0909	Bogra, BD-2011
Hpms 2-21		
300	0.0455	Pepsicum
304	0.1364	BD-2025, Boro, Altaf
308	0.5	Bogra, Balijuri, Tinn Tahori, BD-2011, BD-2035, BD-2043, BD-2082, Angoor, Kamranga, Kalo
311	0.1818	Comilla, Ruma, Bindi
315	0.1364	Dhani, BD-2005, Shada

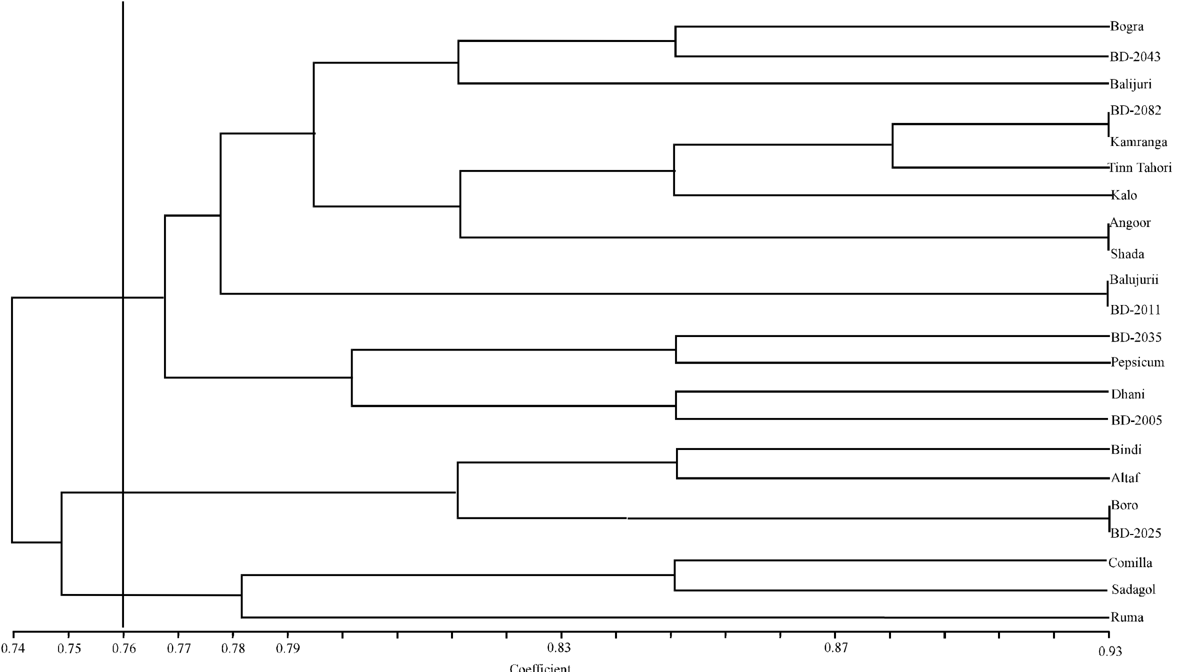

Fig. 5: The UPGMA dendrogram based on Nei (1972) genetic distance summarizing the data on differentiation between 22 chilli germplasm according to SSR analysis

BD-2043, Balijuri morich, BD-2082, Kamranga morich, Tinn Tahori morich, Kalo morich, Angoor morich, Shada morich, Balujurii morich, BD-2011, BD-2035, BD-2005, Pepsicum morich, Dhani morich formed cluster 1 in which

Table 4: Summary of Nei (1972) genetic distance values for different cultivar pairs of chilli

Variety	Bogra	BD-2035	BD-2043	BD-2082	Bindi	Boro	Angoor	Shada	Altaf	Kam ranga	Pepsicum	Comilla	Kalo	Balujurii	Sada gol	Ruma	Balijuri	Dhani	Tinn Tahori	BD-2005	BD-2011
BD-2035	0.778																				
BD-2043	0.852	0.852																			
BD-2082	0.778	0.852	0.852																		
Bindi	0.778	0.704	0.704	0.704																	
Boro	0.704	0.778	0.778	0.852	0.778																
Angoor	0.778	0.778	0.778	0.852	0.778	0.852															
Shada	0.704	0.704	0.704	0.778	0.778	0.852	0.926														
Altaf	0.704	0.704	0.704	0.704	0.852	0.852	0.778	0.778													
Kamranga	0.778	0.778	0.778	0.926	0.704	0.778	0.926	0.852	0.704												
Pepsicum	0.704	0.852	0.778	0.852	0.704	0.852	0.778	0.778	0.704	0.778											
Comilla	0.704	0.778	0.852	0.778	0.778	0.778	0.704	0.704	0.704	0.704	0.778										
Kalo	0.852	0.778	0.778	0.852	0.778	0.704	0.778	0.704	0.704	0.852	0.704	0.704									
Balujurii	0.778	0.852	0.778	0.778	0.704	0.704	0.778	0.704	0.778	0.778	0.778	0.704	0.778								
Sada gol	0.704	0.704	0.778	0.704	0.778	0.704	0.704	0.704	0.778	0.704	0.704	0.852	0.704	0.778							
Ruma	0.778	0.778	0.704	0.704	0.852	0.704	0.704	0.704	0.704	0.704	0.778	0.778	0.778	0.778	0.778						
Balijuri	0.778	0.778	0.852	0.778	0.704	0.704	0.852	0.778	0.704	0.852	0.704	0.778	0.778	0.778	0.778	0.704					
Dhani	0.704	0.778	0.852	0.852	0.704	0.852	0.778	0.852	0.704	0.778	0.852	0.852	0.704	0.704	0.778	0.704	0.778				
Tinn Tahori	0.778	0.778	0.778	0.852	0.704	0.704	0.852	0.778	0.704	0.926	0.704	0.704	0.852	0.778	0.704	0.704	0.852	0.704			
BD-2005	0.704	0.778	0.778	0.852	0.704	0.778	0.704	0.778	0.704	0.778	0.778	0.778	0.778	0.704	0.704	0.704	0.704	0.852	0.778		
BD-2011	0.852	0.778	0.778	0.778	0.704	0.704	0.778	0.704	0.778	0.778	0.704	0.704	0.778	0.926	0.778	0.704	0.778	0.704	0.778	0.704	
BD-2025	0.704	0.778	0.778	0.778	0.778	0.926	0.778	0.778	0.852	0.704	0.778	0.778	0.704	0.704	0.704	0.704	0.704	0.778	0.704	0.778	0.704

sub cluster I includes Bogra morich, BD-2043, Balijuri morich, BD-2082, Kamranga morich, Tinn Tahori morich, Kalo morich, Angoor morich, Shada morich, Balujurii morich, BD-2011 and sub cluster II includes BD-2035, BD-2005, Pepsicum morich, Dhani morich.

Bindi morich, Altaf morich, Boro morich, BD-2025 were grouped in cluster 2 in which sub cluster-I includes Bindi morich, Altaf morich and sub cluster II includes Boro morich and BD-2025.

In cluster 3, Comilla morich and Sada gol morich formed sub cluster I and Ruma morich formed sub cluster-II.

From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type formed cluster together. Maximum PGRC chilli germplasms formed in cluster 1 and Ruma morich alone made sub cluster-II of cluster 3.

Thus microsatellite markers could identify the chilli genotypes and some of the chilli genotypes under investigation have probably originated from closely related ancestors and possess high degree of genetic similarity.

CONCLUSION

The result of the present study would be useful to know genetic variation, population structure, parentage assessment, genome mapping, marker assisted selection (MAS), varieties purity, etc of different chilli. This information would identify distinct chilli groups existing in Bangladesh and consequently enables a genetic conservation plan.

Higher genetic variability within populations and significant genetic differentiation between populations indicate rich genetic resources of a species. It also indicated that these varieties were derived from different origin and could be utilized in breeding programme for traits of interest. SSR markers have been proved to be powerful tool for molecular genetic analysis of chilli cultivars; Angoor morich, Shada morich, BD-2082, Kamranga morich, Tinn Tahori morich; Balujurii morich, BD-201, Boro morich and BD-2025 could be utilized in hybridization programme to assess genetic diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand with biotic and abiotic factors.

ACKNOWLEDGMENTS

We would like to acknowledge the BAS_USDA (Bangladesh Academy of Science-United State Department of Agriculture) project (Proposal Identification No. CR10), entitled “Selection for Anthracnose Resistance in Chilli Germplasm and Development of Resistant Line” for the financial and technical support. Special thanks are conveyed to the Bangladesh Institute of Nuclear Agriculture (BINA) for providing funding for molecular work.

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