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GENETIC DIVERSITY ASSESSMENT BY MICROSATELLITE MARKERS IN SUMMER CHILLI (*Capsicum annuum* L.) GENOTYPES OF BANGLADESH

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ABSTRACT

Assessment of genetic diversity among different is a prerequisite for plant breeders in choosing potential parental lines. Ten genotypes of summer growing chilli including one released variety viz., BARI morich-2; one advanced line viz., SRC- 517 and eight local cultivars viz., IAH-160, IAH-164, AHM-206, AHM-217, AC-63, AC-312, RAI-67, and RI-1(6) were characterized with a view to explore genetic diversity within these genotypes based molecular markers. Using eight microsatellite (SSR) primers across 10 genotypes, a total of 30 alleles with an average number of 3.75 alleles per locus were found. The number of alleles detected varied form three (CAMS-864, CAMS-880, CAMS-885) to five (CAMS-647). The allele size ranged from 160 (CAMS-075) to 289 bp (CAMS-864). All most all sets of primers showed high polymorphism (PIC value ≥0.6) except CAMS-880 and CAMS-885 which suggesting the greater genetic diversity in the genotypes. Gene diversity ranged from 0.46 (CAMS-885) to 0.74 (CAMS-647) and the highest and lowest value of Shannon's Information Index was registered in the same locus with their average value of 1.174. Higher level of genetic differentiation (0.971) and lower level of gene flow value (0.007) which were indicative of the presence of diversity among the genotypes. The genotypes had distinct status in the dendrogram, because of variation in genetic distance values differed from 0.138 to 0.938.

Keywords: Summer Chilli, Genetic diversity, SSR marker, Polymorphism, Dendrogram.

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INTRODUCTION

Chilli (*Capsicum annuum* L., 2n = 24) a member of the Solanaceae family has originated from South and Central America. It is an indispensable spice due to its pungency, taste, appealing colour and flavor. It is available and used in human food preparation in the forms of green, dried and powdered. Chilli is a valuable spice and also one of the most important cash crops grown in Bangladesh. It has become an essential ingredient in Bangladeshi dietary patterns. It is cultivated in both the summer and winter seasons. A number of cultivars are grown in Bangladesh differing in habit, yield, consumer's preference and in size, shape, colour and pungency of the fruit (Farhad et al., 2010). The area and production of Kharif (April to September) chilli was 20,506 ha and 99,126 MT, respectively; while 79,629 ha and 393,556 MT were recorded in Rabi (October to March) chilli, respectively. The yield was around 1.68 MT/ha (BBS, 2021).

Although a good number of winter growing genotypes have been cultivated in different parts of Bangladesh, very limited numbers of genotypes are available for cultivation in summer season. It is one of the major reasons to cultivate in limited area as well as lower production compared to winter season. Apart from developing traditional varieties through conventional breeding, exploitation of heterosis for yield and yield attributing characters through hybridization is also important in crop improvement. Screening of available germplasm helps in studying the variability and diversity and identification of superior parents for use in hybridization. Genetic divergence existing in the population helps in the selection of suitable parents for utilization in any crop breeding programme leading to reduction in the number of crosses (Guerra et al., 1999). Previously genetic diversity in *Capsicum* was studied using morphological, cytological and biochemical markers (Kaur and Kapoor, 2001; Gopinath et al., 2006). Recent developments in DNA based technologies have revolutionized the utilization of molecular markers in Genetics and Breeding studies (Paterson et al., 1991; Rafalski et al., 1996). Hence, assessment of genetic diversity is more meaningful when it can be assessed through molecular polymorphism along with at the phenotypic level as the later involves data on morphological traits which are environmental dependent.

Of all classes of DNA based marker, the microsatellite SSR (simple sequence repeat) is polymerase chain reaction (PCR) based, highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible, and randomly and widely distributed in the genome (Powell et al., 1996). Furthermore, simple sequence repeats (SSRs) are the most widely used marker system for plant variety characterization and diversity analysis especially in cultivated species which have low levels of polymorphism (Dhaliwal et al., 2014). DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of plant lines. Moreover, the analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising et al., 1995). For characterization and documentation, distinct

morphological along with molecular has been used in 20 crop species including rice (*Oryza sativa* L.), wheat (*Triticum aestivum*), maize (*Zea mays* L.), barley (*Hordeum vulgare*), rapeseed (*Brassica napus* L.), soybean (*Glycine max* L.), potato (*Solanum tuberosum* L.) and other crops by Rahman et al. (2006). In Bangladesh, nine soybean cultivars were identified by microsatellite markers, which have provided identity and might work as protection (Islam et al., 2007). Thirteen maize cultivars were also characterized using microsatellite fingerprinting in combination with distinctness, uniformity and stability (DUS) test (Molla et al., 2007) and 94 rice cultivars (Rahman et al., 2008). Based on that experience, the present study has been designed with 10 genotypes of summer chilli using the morpho-molecular traits i) to analyze genetic diversity and relationship among the genotypes, and ii) to identify distinct morphological characteristics along with establish allelic patterns to generate a reference database to support cultivar protection and settle possible commercial disputes as well as to guide breeding programmes and genetic resources of the species.

MATERIALS AND METHODS

Plant samples and extraction of genomic DNA

Molecular diversity using SSR markers has been studied at the Molecular Biology Lab., Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur. A total of 10 genotypes of summer growing chilli genotypes representing different geographical distribution were selected for the present study (Table 1). The genomic DNA was isolated from a bulk of three week old seedling leaf tissues taken from five plants from each genotype using SDS (Sodium dodecyl sulfate) and phenol: chloroform: IAA followed by alcohol precipitation described by Saghai-Maroof et al. (1984) and also used by Rahman et al. (2007) with some modifications.

Quantification and optimization of DNA concentration

Presence of genomic DNA was confirmed on 1% agarose gel qualitatively. The gels were visualized under UV light and photographed using photo documentation system (UV Transilluminator, Uvitec, UK). All of the DNA samples were found to be in good quality in this study. The amount of genomic DNA was quantified using UV a spectrophotometer (Spectronic® GENESYS™ 10 Bio) at 260 nm. Using the absorbance reading the original DNA concentrations were determined according to the following equation:

Before PCR amplification of DNA, the DNA concentrations were adjusted to 25 ng μl^{-1} using the following formula: $S_1 \times V_1 = S_2 \times V_2$ Where, S_1= Initial strength (ng μl^{-1}), V_1= Initial volume (µl), S_2= Final strength (ng μl^{-1}) and V_2= Final volume (µl)

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

Table 1. List of summer growing chilli genotypes used in this study with their collection sites in Bangladesh.

Sl. No.	Genotypes	Location of collecting site (Upazila and District)	Latitude (N)	Latitude (E)
01	IAH-160	Sadar, Gazipur	24° 0′	90° 25.30′
02	IAH-164	Kaliakoir, Gazipur	24° 4.30′	90° 13.0′
03	AHM-206	Dhamrai, Dhaka	23° 54.30′	90° 13.0′
04	AHM-217	Dhamrai, Dhaka	23° 54.30′	90° 13.0′
05	AC-63	Shakhipur, Tangail	24° 19.9′	90° 10.20′
06	AC-312	Kaliganj, Gazipur	23° 55.30′	90° 34.01′
07	RAI-67	Hathazari, Chittagong	22° 30.13′	91° 48.27′
08	SRC-517	RSRC, BARI, Gazipur	24° 22.8′	88° 39.42′
09	BARI morich-2	RSRC, BARI, Gazipur	24° 22.8′	88° 39.42′
10	RI-1(6)	Ramgarh, Khagrachori	22° 58.0′	91° 42.0′

Identification and selection of microsatellite or SSR primers

Preliminarily, 50 microsatellite primer pairs were tested to identify discriminating alleles those are located in 12 chromosomes of chilli from different publications. Among them 39 were selected for their better responsiveness with clear and expected amplified product sizes which were used for microsatellite analysis in the present study (Table 2).

PCR standardization and amplification

The Polymerase chain reactions was set up 10 µl volumes containing 50 ng template DNA, 5X Green GoTaq® Reaction Buffer included 7.5 mM $MgSO_4$,1.25 U μl^{-1} Taq DNA polymerase, 0.4 mM each of the deoxyribonucleotide triphosphate (dNTPs), 10 µM of each of primer, 0.5% DMSO (dimethyl sulfoxide) and a suitable amount of sterile deionized water. SSRs were amplified under the following "touchdown" PCR conditions: 94°C for 3 min denaturation, 11 cycles of 94°C for 0.5 min, 58-60°C for 1 min, decreasing by 1°C per cycle, and 72°C for 1 min; 30 cycles of 94°C for 0.5 min, 52-55°C for 1 min and 72°C for 1 min; a final extension for 5 min. For checking amplification, the PCR products were electrophoretically resolved on 2% agarose gel in 1X TBE.

Electrophoretic separation and visualization of PCR products

PCR-products were electrophoresed on a 5% denaturing polyacrylamide gel containing 19:1 acrylamide: bis-acrylamide, 10X TBE buffer, 10% APS and ultrapure Temed. Electrophoresis was done using the Triple Wide Mini-Vertical Electrophoresis System, MGV-202-33 (CBS Scientific, USA).

Table 2. List of microsatellite primers used in this study

Sl.	Locus	Primer sequence (5'-3')	Repeat motif	Ann. T.	Chr. no.	Expected Size (bp)	Reference
1	CAMS-075	F: actaattacacattctgcat tttctc R: aggctcgagtaccacgaaga	$(tg)_{10}$	54ºC	5	190	Minamiya ma et al. (2006)
2	CAMS-864	F:ctgttgtggaagaagaggaca R: gcttctttttcaacctcctcct	$(aga)_{32}$	54ºC	7	222	
3	CAMS-880	F: gagccaagaaaaaggtggaa R: caactcatcgttcaacaacaca	(gaa)12	53ºC	6	237	
4	CAMS-065	F: ccagtctcatccagcagaca R: catatgctgctcctgcattc	$(ac)_{12}$	52ºC		213	
5	CAMS-236	F: ttgtagtttgcgtaccatttga R: atgaatccagggttccacaa	(ac)14a (ta)10	54ºC	2	191	
6	CAMS-885	F: aacgaaaaacaaacccaatca R: ttgaaattgctgaaactctgaa	(gaa)28	53ºC	2	248	
7	CAMS-647	F: cggattcggttgagtcgata R: gtgctttggttcggtctttc	(tat)6tg (tta)3… (tat)21	54ºC	3	221	
8	CAMS-855	F: aagtgtcaaggaaggggaca R: cctaaccacccccaaaagtt	(agt)14a (gaa)9	54ºC	8	243	

Ann. T.: Annealing Temperature, Chr. no.: Chromosome number

Scoring and analysis of microsatellite data

A single genotypic data matrix was constructed for all loci. Statistics of genetic variation were calculated using allelic frequency estimates obtained from genotypic frequencies of SSR loci using the computer program POPGENE (Version 1.31) (Yeh et al., 1999). The polymorphism information content (PIC) of the SSR used or gene diversity value was calculated as PIC= 1- ΣX_i^2; Where, X*i* is the frequency of the *i*-th allele of a particular locus. The software DNA FRAG version 3.03 was used to estimate allelic length (Nash, 1991).

RESULTS AND DISCUSSION

Microsatellite polymorphism

Microsatellite polymorphism subjected to 10 summer growing chilli genotypes were successfully amplified with the eight microsatellite primer pairs. Four typical SSR profiles are shown in Fig. 1 (A-D). Analysis of the variability parameters for the eight SSRs in the 10 summer chilli genotypes were shown in Table 1. A total of 30 alleles with an average number of 3.75 alleles per locus were found in the present study. The number of alleles detected varied form three (CAMS-864, CAMS-880, CAMS-885) to five (CAMS-647). The allele sizes ranged from 160 (CAMS-075) to 289 bp (CAMS-864) (Table 3).

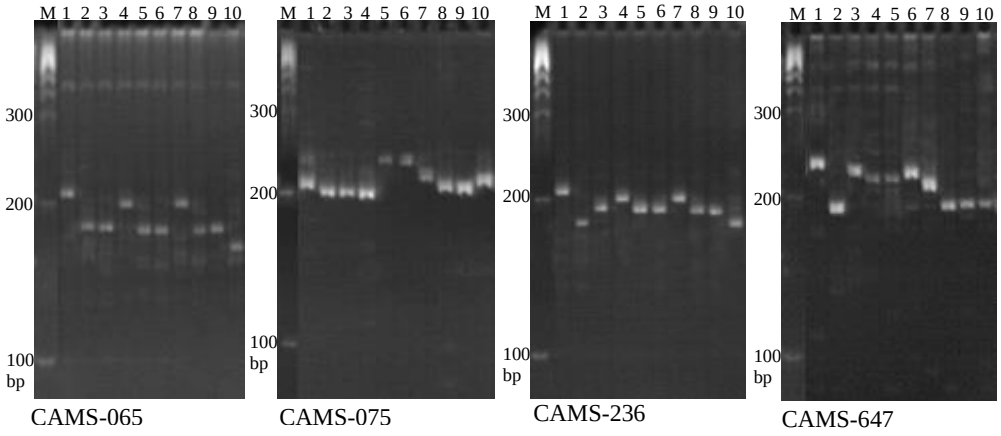

Figure 1. Microsatellite profiles of 10 summer chilli genotypes at locus CAMS-065 (A), CAMS-075 (B), CAMS-236 (C) and CAMS-647 (D); M: Molecular wt. marker (100 bp DNA ladder)

Lane 1: IAH-160, Lane 2: IAH-164, Lane 3: AHM-206, Lane 4: AHM-217, Lane 5: AC-63, Lane 6: AC-312, Lane 7: RAI-67, Lane 8: SRC-517, Lane 9: BARI morich-2, Lane 10: R-1(6)

Hossain et al. (2014) evaluated the genetic diversity within 22 chilli germplasm by using four microsatellite markers and a total of 27 alleles were detected and the number of alleles per marker ranged from 4 to 13 (size range was 153-315 bp). Hanacek et al. (2009) assessed genetic diversity among 41 accessions of red pepper using eight microsatellite markers. The five polymorphic markers were amplified with two to eight alleles per locus. In total, 28 alleles with an average of 3.5 alleles per microsatellite locus were detected. The average numbers of 3.15 and 3.75 alleles per locus of the present study while compared with those of previous studies in chilli showed some variations. The possible reason for the variation might be due to the high number of diverse chilli genotypes used in this study. Observed number of alleles was higher for each locus which can be explained by high number of diverse chilli genotypes used. The observed differences allelic length for each locus indicated the presence of broad

genetic base among the chilli genotypes used in this study. The broad genetic base could be one of the reasons for the high yield of polymorphic markers as reported by Molla et al. (2015).

Table 3. Summary of genetic variation statistics for all loci used for 10 summer chilli genotypes analysis.

Locus	na*	ne*	Major allele frequency	Ho*	He*	Nei*	I*	Fst	Nm*
CAMS-075	4	2.941	0.500	0.000	0.695	0.660	1.221	0.978	0.003
CAMS-864	3	2.941	0.400	0.000	0.695	0.660	1.089	1.000	0.000
CAMS-880	3	2.381	0.500	0.000	0.611	0.580	0.943	1.000	0.000
CAMS-065	4	3.571	0.400	0.000	0.758	0.720	1.332	0.962	0.005
CAMS-236	4	2.941	0.500	0.000	0.695	0.660	1.221	1.000	0.000
CAMS-885	3	1.852	0.700	0.000	0.484	0.460	0.802	0.847	0.040
CAMS-647	5	3.846	0.400	0.000	0.779	0.740	1.471	1.000	0.000
CAMS-855	4	3.571	0.300	0.000	0.758	0.720	1.314	0.984	0.006
Mean	3.750	3.006	0.463	0.000	0.684	0.650	1.174	0.971	0.007

na = Observed number of alleles, ne = Effective number of alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, Nei'* = Nei's (1973) expected heterozygosity, I* = Shannon's Information Index, Nm* = Gene flow estimated from Fst = 0.25 (1 - Fst)/Fst, Fst = Genetic differentiation

Expected heterozygosity (He, average 0.484) values for each SSR locus, considering all studied genotypes, were always higher than the observed heterozygosity (Ho), representing homozygous individuals in population samples. The highest and lowest value of Shannon's Information Index was registered in the locus CAMS-647 and CAMS-885, respectively with their average value of 1.174. Genetic differentiation values were extended between 0.847 and 1 with an average of 0.971 and gene flow values ranged from 0.000 to 0.007 with an average of 0.007 (Table 3). Study results demonstrated comparatively higher level of genetic differentiation and low level of gene flow values in 10 chilli genotypes were indicative of diversity among the genotypes as most of the studied genotypes were of local origin/cultivars.

The higher level of gene diversity [Nei's (1973) expected heterozygosity, average 0.650] as observed in the present study has also been reported by Hossain et al. (2014) in chilli and in other crops viz., brinjal (Rahman et al., 2010), mung bean (Molla et al., 2016) and musk melon (Molla et al., 2017). The similarities of present study results with that of others might probably be due to the greater diversity of genotypes used in the present study.

The PIC values for eight primers across 10 summer genotypes was obtained in the present study varied from 0.460 for CAMS-647 to 0.740 for CAMS-885, with an

Table 4. Size and frequency of alleles and diversity index at eight SSR loci across 10 summer chilli genotypes

Locus	Repeat Motif	Allele sizes (bp)	Average allele sizes (bp)	Allele frequency	PIC
CAMS-075	$(tg)_{10}$	207	184	0.100	0.660
		196		0.200	
		174		0.500	
		160		0.200	
CAMS-864	$(aga)_{32}$	289	254	0.300	0.660
		241		0.400	
		232		0.300	
CAMS-880	$(gaa)_{12}$	228	217	0.100	0.580
		217		0.500	
		205		0.400	
CAMS-065	$(ac)_{12}$	237	216	0.200	0.720
		216		0.200	
		207		0.200	
		202		0.400	
CAMS-236	$(ac)_{14}a(ta)_{10}$	209	195	0.100	0.660
		200		0.200	
		192		0.500	
		180		0.200	
CAMS-885	$(gaa)_{28}$	245	225	0.200	0.460
		219		0.700	
		211		0.100	
CAMS-647	$(tat)_6tg(tta)_{3...}(tat)_{21}$	240	216	0.100	0.740
		227		0.200	
		216		0.200	
		209		0.100	
		188		0.400	
CAMS-855	$(gaa)_{28}$	288	255	0.300	0.720
		255		0.100	
		243		0.300	
		233		0.300	

average value of 0.65. Allele frequency ranged from 0.1 to 0.7 with an average value of 0.267 (Table 4). All studied SSRs were polymorphic among chilli genotypes and informative for describing their genotypic variation (i.e., PIC values different from zero) (Table 4). Six of these SSRs were very informative with higher PIC values (>0.6) which was in accordance with the previous findings reported by Lee et. al. (2004), Minamiyama et al. (2006) and Mimura et al. (2012). Lower PIC values indicate the presence of closely related genotypes; while higher PIC values indicate the presence of diverse genotypes. High PIC values as observed might be due to use of di-nucleotide repeats and also due to genotypic differences as reported by Molla et al. (2010).

Nei's genetic distance between the genotypes

Over all Nei's genetic distance value ranged from 0.138 to 0.998 among 45 pairs resulting as a means of permutation combination of 10 summer chilli genotypes. Higher genetic distance values (>0.6) observed across 37 pairs where 12 pairs (IAH-160 vs IAH-164, AC-312, BARI morich-2, RI-1(6), RAI-67 vs IAH-164, AC-63, AC-312, RI-1(6) vs AHM-206, AC-63, AHM-217 vs AHM-206, AC-312 and BARI morich-2 vs AC-312) yielded the highest and two pairs (RAI-67 vs BARI morich-2 and SRC-517) yielded the lowest genetic distance values. Others highest and lowest genetic distance values can be seen in the Table 5.

Table 5. List of genotype pairs of summer chilli showed higher and lower values of Nei's (1972) genetic distance

Sl.no.	Genotype pair			Genetic Distance (*D*)	Sl.no.	Genotype pair			Genetic Distance (*D*)
1	IAH-164	vs	IAH-160	0.998	24	BARI morich-2	vs	AC-63	0.803
2	AC-312	vs	IAH-160	0.998	25	SRC-517	vs	AC-312	0.803
3	BARI morich-2	vs	IAH-160	0.998	26	RI-1(6)	vs	AC-312	0.803
4	RI-1(6)	vs	IAH-160	0.998	27	RI-1(6)	vs	RAI-67	0.803
5	RAI-67	vs	IAH-164	0.998	28	RI-1(6)	vs	BARI morich-2	0.609
6	AHM-217	vs	AHM-206	0.998	29	RAI-67	vs	IAH-160	0.609
7	RI-1(6)	vs	AHM-206	0.998	30	AHM-206	vs	IAH-164	0.609
8	AC-312	vs	AHM-217	0.998	31	AC-63	vs	AHM-217	0.609
9	RAI-67	vs	AC-63	0.998	32	RAI-67	vs	AHM-217	0.609
10	RI-1(6)	vs	AC-63	0.998	33	SRC-517	vs	AHM-217	0.609
11	RAI-67	vs	AC-312	0.998	34	BARI morich-2	vs	AHM-217	0.609
12	BARI morich-2	vs	AC-312	0.998	35	RI-1(6)	vs	AHM-217	0.609
13	AHM-206	vs	IAH-160	0.803	36	SRC-517	vs	AC-63	0.609
14	AHM-217	vs	IAH-160	0.803	37	RI-1(6)	vs	SRC-517	0.609
15	AC-63	vs	IAH-160	0.803	38	AC-63	vs	IAH-164	0.471

Sl.no.	Genotype pair			Genetic Distance (*D*)	Sl.no.	Genotype pair			Genetic Distance (*D*)
16	SRC-517	vs	IAH-160	0.803	39	RI-1(6)	vs	IAH-164	0.471
17	AHM-217	vs	IAH-164	0.803	40	AC-312	vs	AHM-206	0.471
18	SRC-517	vs	IAH-164	0.803	41	AC-312	vs	IAH-164	0.364
19	BARI morich-2	vs	IAH-164	0.803	42	BARI morich-2	vs	SRC-517	0.364
20	AC-63	vs	AHM-206	0.803	43	AC-312	vs	AC-63	0.276
21	RAI-67	vs	AHM-206	0.803	44	SRC-517	vs	RAI-67	0.138
22	SRC-517	vs	AHM-206	0.803	45	BARI morich-2	vs	RAI-67	0.138
23	BARI morich-2	vs	AHM-206	0.803	-	-	-	-	-

From the difference between the highest and the lowest genetic distance values it was revealed that there was wide variability among studied chilli genotypes. However, closeness may be possible in the genetic makeup of the locus for which the primers were responsible to distinguish along with low variation also in the morphological traits and geographical sources. The highest genetic distance can be explained by the fact that local cultivars or land races collected from different location have been included in the study. The existing distance can further be used for inclusion of gene sources from the traditional varieties to HYVs, using the genetic fingerprinting and correlating the values with that of the morpho-physiological traits to find out the best performing varieties through appropriate breeding programmes. Information on variability expression rate through genetic distance based on morphological traits and geographical origin was also reported in previous investigations conducted by Rahman et al. (2010), Hossain et al. (2014), Molla et al. (2016) and Molla et al. (2017).

Phylogenetic dendogram

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendogram depicted of 10 summer chilli genotypes based Nei's (1972) genetic distance. The studied genotypes segregated generally into two main groups in where six genotypes gathered in group "A" while other four genotypes in group "B" (Fig. 2). All of 10 summer chilli genotypes disseminated in different sub-clusters which might be possible due to higher genetic distance value (>0.6) as were observed in between 82% genotypic pairs (Table 5). It was noticed that the highest genetic distance presenting genotypic pairs separated in different sub-clusters (Fig. 2 and Table 5). As for instance, IAH-160 vs. IAH-164, AC-312, BARI morich-2, RI-1(6) and RAI-67 got the highest genetic distance (Table 5) and in this aspect their geographical source was different with distinct morphological states like IAH-160 showed unique descriptor states regarding stem colour before and after transplanting as well as filament colour (Table

6). Moreover, geographical source was Sadar, Gazipur for the genotype IAH-160 whereas RAI-67 and RI-1(6) was originated from Hathazari, Chittagong and Ramgarh, Khagrachori, respectively (Table 1). On the other hand, RAI-67, SRC-517 and BARI morich-2 were grouped in same cluster because of lower genetic distance (0.138) as were observed between RAI-67 vs. SRC-517 and BARI morich-2 (Table 5). As BARI morich-2 is a released variety, there are possibilities that the farmers used to collect the BARI morich-2 and grew in their areas with some modifications of the traits and or the collected source genetic materials of BARI morich-2 could have originally came from that base source which was close to the other one. This is why the morphological variation was evident among the three genotypes (Table 6). Results of the present study and those reported by Rahman et al. (2010), Hossain et al. (2014), Molla et al. (2016) and Molla et al. (2017) suggested that genetic distance value separated the genotypes in different sub-clusters where such values depend on their morphological characters as got selected in different geographical locations.

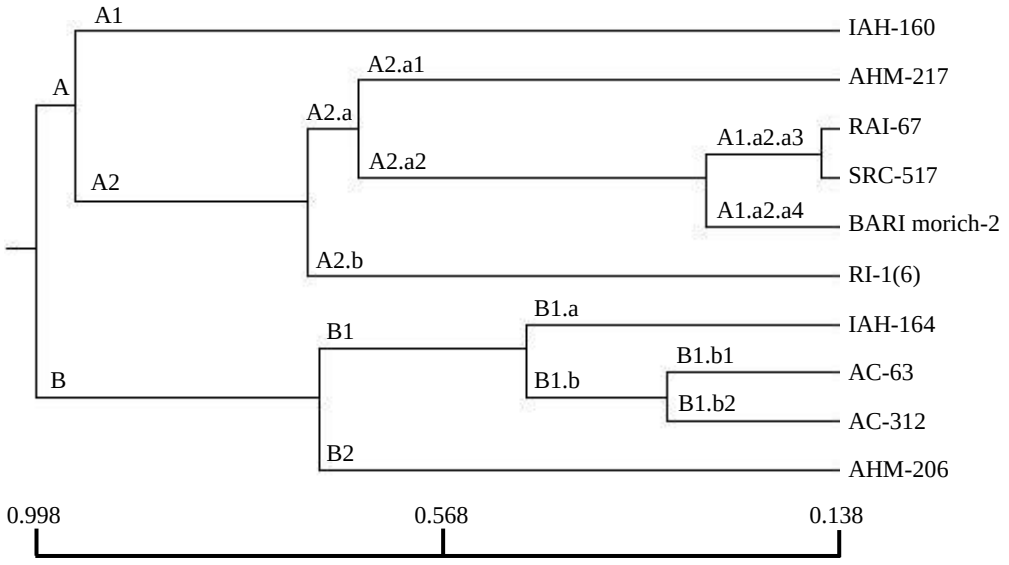

Figure 2. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between 10 summer chilli genotypes according to microsatellite analysis

Table 6. Distinctness of 10 summer chilli genotypes based on qualitative traits

Sl. no.	Genotype	Leaf shape	Leaf color	Filament color	Corolla color	Fruit shape	Fruit position
1	BARI morich-2	Ovate	Green	White	Light yellow	Triangular	Inserted
2	SRC-517	Ovate	Dark green	White	Light yellow	Elongate	Same level
3	AHM-217	Ovate	Purple	White	White	Elongate	Same level
4	RAI-67	Ovate	Purple	White	Light yellow	Elongate	Same level
5	AC-63	Ovate	Purple	White	White	Elongate	Same level
6	AC-312	Ovate	Dark green	White	White with purple margin	Elongate	Exerted
7	IAH-160	Deltoid	Purple	Purple	Light yellow	Triangular	Exerted
8	AHM-206	Lanceolate	Purple green	White	White	Elongate	Exerted
9	IAH-164	Ovate	Purple	White	White	Elongate	Same level
10	RI-1(6)	Ovate	Purple green	White	White	Elongate	Inserted

CONCLUSION

It can be concluded from this study that a comparative evaluation of the repeatability of molecular markers has been performed in order to ascertain the genetic diversity among summer growing chilli genotypes in Bangladesh. The study also indicated that 10 genotypes derived from different origin which were identified as diversified and could be utilized in breeding programme for traits of interest. SSR markers have proven to be effective tools for molecular genetic analysis of chilli cultivars for plant breeding programs in order to evaluate the existing genetic diversity.

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