doi:10.14720/aas.2022.118.4.2511 Original research article / izvirni znanstveni članek

Genetic diversity in - chilli (*Capsicum annuum* L.) based on microsatellite markers: An evaluation of Bangladeshi germplasm

Md. Rezwan MOLLA [1, 2], Iftekhar AHMED [1, 2], Md. Motiar ROHMAN [3], Mohammad Amdadul HAQUE [4, 5, 6], Shah Md. Monir HOSSAIN [7], Lutful HASSAN [2]

Received January 16, 2022; accepted September 23, 2022.
Delo je prispelo 16. januarja 2022, sprejeto 23. septembra 2022

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Abstract: Genetic diversity analysis is a pre-requisite to develop improve variety of any crop. Hence, 39 SSR markers were used to analyze the genetic diversity of local chilli cultivars. PCR-amplified microsatellite loci were shown to be polymorphic in all investigated cultivars. The locus, CAMS-647 produced the highest number of alleles (8) ranging in size from 188 to 279 bp. PIC values for 39 primers ranged from 0.099 for the locus Hpms 1-165 to 0.806 for the locus CAMS-679. All of the SSRs examined were informative in characterizing the genotypic variance of the samples while 12 were more informative with higher PIC values (> 0.6). There was a wide range of genetic diversity varied from 0.117 (HpmsE075) to 0.806 (CAMS-647), whereas the highest (1.713) and the lowest (0.205) value of Shannon's Information Index was registered in the locus CAMS-679 and Hpms 1-165, respectively. There was a higher degree of genetic differentiation (0.927) and a lower amount of gene flow (0.010). Nei's genetic distance (GD) varied from 0.100 to 0.990. Among 96 cultivars, 55 had distinct status in the dendrogram with higher GD values (> 0.6), while 41 cultivars showed a close relationship and yielded lower GD values.

Key words: chilli; genetic diversity; microsatellite (SSR) markers; polymorphism information content

Določanje genetske raznolikosti čilija (*Capsicum annuum* L.) z mikrosateliti: Ovrednotenje genetskega materiala v Bangladešu

Izvleček: Analiza genetske raznolikosti je predpogoj za vzgojo izboljšanih sort katerekoli kulturne rastline. Zatradi tega je bilo uporabljenih 39 SSR lokusov za analizo genetske raznolikosti genotipov čilija. S PCR pomnoženi mikrosatelitski lokusi so bili polimorfni pri vseh preučenih genotipih. Pri lokusu CAMS-647 smo zaznali največje število alelov (8), ki so obsegali dolžine od 188 do 279 bp. PIC vrednosti so za 39 začetnih oligonukleotidov (primerjev) znašale od 0,099 za lokus Hpms 1-165 do 0,806 za lokus CAMS-679. Vsi analizirani mikrosateliti (SSR) so bili za vrednotenje genenotipske variabilnosti vzorcev informativni, med njimi jih je bilo 12 z večjimi PIC vrednostmi (> 0,6) najprimernejših. Genetska raznolikost je bila velika in je variirala od 0,117 (HpmsE075) do 0,806 (CAMS-647), največja (1,713) in najmanjša (0,205) vrednost Shannonovega informacijskega indeksa sta bili ugotovljeni za lokusa CAMS-679 in Hpms 1-165. Ugotovljena je bila visoka stopnja genetske diferenciacije (0,927) in majhen pretok genov (0,010). Neijeva genetska distanca je variirala med 0,100 in 0,990. Med 96 genotipi jih je imelo 55 jasen položaj v dendrogramu z večjimi vrednostmi genske distance (> 0,6) medtem, ko je 41 genotipov pokazalo ožjo sorodnost z manjšimi v rednostmi genske distance.

Ključne besede: čili; genetska raznolikost; microsatelistki markerji (SSR); informacijska vrednost polimorfizma

1 Bangladesh Agricultural Research Institute, Plant Genetic Resources Centre, Gazipur, Bangladesh
2 Bangladesh Agricultural University, Faculty of Agriculture, Department of Genetics and Plant Breeding, Mymensingh, Bangladesh
3 Bangladesh Agricultural Research Institute, Plant Breeding Division, Gazipur, Bangladesh
4 Universiti Putra Malaysia, Faculty of Agriculture, Department of Crop Science, Serdang, Selangor, Malaysia
5 Bangladesh Agricultural Research Institute, Horticulture Research Centre, Gazipur, Bangladesh
6 Corresponding author, e-mail: amdad80@gmail.com
7 Bangladesh Agricultural Research Council, Crops Division, Dhaka, Bangladesh

1 INTRODUCTION

Chilli (*Capsicum* spp.) belongs to the Solanaceae family, having chromosome number 2n = 2x = 24 (Sharmin et al., 2018)with a mean of 2.00 alleles per primer. Gene diversity ranged from 0.333 to 1.00 with an average of 0.567. Polymorphic Information Content (PIC. The genus is native to Central and South America (Pickersgill, 1991) which includes five species viz., *Capsicum chinense* Jacq., *Capsicum baccatum* L., *Capsicum frutescens* L., *Capsicum pubescens* Ruiz & Pav. and *Capsicum annuum*. Of these, *C. annuum* is the most important one because of its versatile use and is cultivated in both tropical and temperate areas in the world. It is used as vegetables, spice, colorant, and for some medical applications (Hernández-Pérez et al., 2020). Chilli is a valuable spice and one of the most important cash crops grown in Bangladesh. It is available and used in human food preparation in the forms of green, dried and powdered. It has become an essential ingredient in Bangladeshi dietary patterns. A number of cultivars are grown in Bangladesh showing differences in growth habit, size, shape, color, pungency, and yield indicating the presence of wider genetic variation (Farhad et al., 2010). The area and production of Kharif (April to September) chilli was 19,000 ha and 36,000 mt, respectively; while 83,000 ha and 105,000 mt were recorded in Rabi (October to March) chilli, respectively. The average yield was around 1.68 mt ha^{-1} (BBS, 2021). Bangladesh has a high diversity of cultivars belonging to various cultivated chilli varieties. Due to the long history of cultivation, selection and popularity of crops, sufficient genetic variability has been generated. 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 (van Zonneveld et al., 2012). As the country has vast chilli resources, and the demand is using those for further development of new materials that can provide a high economic return. Hence, it is necessary to study some basic traits of similarity and/or parent progeny relationship that can indicate their adoption in the country. Selection of useful diversity from the available genetic resources will be an enormous challenge. Collection and maintenance of the genetic diversity in chilli are important to avoid genetic erosion. Besides the identification of species, the characterization and evaluation of cultivars maintained in gene banks are of fundamental importance (van Zonneveld et al., 2012). Traditionally, morphological markers known as descriptors have been utilized in plants for varietal identification and genetic diversity study, which is expensive, time-consuming, requiring huge areas of land, and specialized staff, and is subject to variation owing to environmental factors (Molla et al., 2017). However, in elite germplasm, the level of polymorphism for morphological traits is sometimes too low and insufficient to allow for variety/genotype discrimination (Dhaliwal et al., 2014). The DNA marker provides a one-stop solution in this case. Different molecular markers for pepper have been established in the previous decade or so. Microsatellite SSR (simple sequence repeat) is a DNA-based marker that based on PCR, multi-allelic, highly polymorphic, commonly co-dominant, highly repeatable, randomly and extensively distributed across the genome (Jain et al., 2014). Furthermore, SSRs are the most extensively used marker system for identifying plant varieties and analyzing diversity, particularly in cultivated species with low levels of polymorphism (Anumalla et al., 2015). Although some research has been conducted regarding chilli diversity in Bangladesh, inadequate information was generated because of the limited number of cultivars assessed with a limited number of primers. For instance, 20 local chilli cultivars were evaluated using 11 SSR makers by Sharmin et al. (2018)with a mean of 2.00 alleles per primer. Gene diversity ranged from 0.333 to 1.00 with an average of 0.567. Polymorphic Information Content (PIC and 22 cultivars using four microsatellite markers by Hossain et al. (2014). The present study was, therefore, undertaken to estimate genetic diversity of 96 winter chilli cultivars collected from diverse locations of Bangladesh by means of 39 microsatellite markers to guide genetic improvement and to promote increased utilization.

2 MATERIALS AND METHODS

2.1 COLLECTION AND EXTRACTION OF GENOMIC DNA FROM A PLANT SAMPLE

A total of 96 local cultivars (Table 1) of winter growing chilli (*Capsicum annuum* L. representing different geographical distributions were nominated and collected at Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI) for the current study to investigate molecular diversity by using SSR marker. Seeds of collected cultivars were sown on small plastic pots to grow seedlings. For DNA extraction, we used young, fresh, disease- and insect-free leaves. SDS (Sodium dodecyl sulfate), phenol: chloroform: IAA followed by alcoholic precipitation were used to isolate genomic DNA from the leaf tissue of three-week-old seedlings described by Saghai-Maroof et al. (1984) with some modifications. Excluding the usage of liquid nitrogen, the modified protocol included digestion with homogenization buffer [Solution: Tris-50 mM, ethylene di-

Table 1: List of winter growing local chilli cultivars used in molecular characterization with their collection sites in Bangladesh

Sl. No.	Cultivars	Location of collecting site (Upazila and District)	Latitude (N)	Longitude (E)
01	BD-10878	Kazipur, Sirajganj	24° 41.516′	89° 42.83′
02	BD-10879	Galachipa, Patuakhali	22° 9.8′	90° 25.8′
03	BD-10880	Kazipur, Sirajganj	24° 41.711′	89° 43.059′
04	BD-10881	Kazipur, Sirajganj	24° 41.711′	89° 43.059′
05	BD-10882	Kazipur, Sirajganj	24° 41.711′	89° 43.059′
06	BD-10883	Kazipur, Sirajganj	24° 41.925′	89° 42.978′
07	BD-10884	Sadar, Sirajganj	24° 31.511′	89° 40.982′
08	BD-10885	Sadar, Sirajganj	24° 32.671′	89° 40.560′
09	BD-10886	Sadar, Sirajganj	24° 32.671′	89° 40.560′
10	BD-10887	Kalapara, Patuakhali	21° 58.918′	90° 13.60′
11	BD-10888	Kalapara, Patuakhali	21° 58.918′	90° 13.60′
12	BD-10892	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
13	BD-10894	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
14	BD-10895	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
15	BD-10896	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
16	BD-10897	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
17	BD-10898	Galachipa, Patuakhali	22° 9.48′	90° 25.48′
18	BD-10938	Muksudpur, Gopalganj	23° 19.0′	89° 52.0′
19	BD-10934	Dohazari, Chittagong	22° 9.46′	92° 4.22′
20	BD-10935	Dohazari, Chittagong	22° 9.46′	92° 4.22′
21	BD-10936	Dohazari, Chittagong	22° 9.46′	92° 4.22′
22	BD-10913	Kotalipara, Gopalganj	22° 59.0′	89° 59.30′
23	KASI-49	Kotalipara, Gopalganj	22° 59.0′	89° 59.30′
24	BD-10916	Kashiani, Gopalganj	23° 17.618′	89° 47.259′
25	BD-10917	Daulatkhan, Bhola	22° 36.24′	90° 44.60′
26	BD-10918	Daulatkhan, Bhola	22° 36.24′	90° 44.60′
27	BD-10919	Sadar, Bhola	22°37.517′	90° 38.062′
28	BD-10920	Sadar, Bhola	22° 37.517′	90° 38.062′
29	RISA-23	Sadar, Bhola	22° 37.517′	90° 38.062′
30	BD-10921	Sadar, Bhola	22° 37.517′	90° 38.062′
31	BD-10922	Sadar, Bhola	22° 37.517′	90° 38.062′
32	BD-10923	Charfashion, Bhola	22° 11.60′	90° 45.48′
33	BD-10924	Charfashion, Bhola	22° 11.60′	90° 45.48′
34	BD-10925	Charfashion, Bhola	22° 11.60′	90° 45.48′
35	BD-10927	Charfashion, Bhola	22° 11.60′	90° 45.48′
36	BD-10928	Charfashion, Bhola	22° 11.60′	90° 45.48′
37	BD-10929	Charfashion, Bhola	22° 11.60′	90° 45.48′
38	BD-10930	Daulatkhan, Bhola	22° 36.24′	90° 44.60′
39	BD-10931	Daulatkhan, Bhola	22° 36.24′	90° 44.60′

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40	BD-10932	Borhanuddin, Bhola	22° 30′	90° 43.3′
41	BD-10933	Borhanuddin, Bhola	22° 30′	90° 43.3′
42	BD-10900	Madarganj, Jamalpur	24° 54.490′	89° 43.075′
43	BD-10903	Melando, Jamalpur	24° 56.962′	89° 52.622′
44	BD-10904	Melando, Jamalpur	24° 56.962′	89° 52.622′
45	BD-10905	Melando, Jamalpur	24° 56.962′	89° 52.622′
46	BD-10906	Melando, Jamalpur	24° 56.962′	89° 52.622′
47	RT-09	Melando, Jamalpur	24° 56.962′	89° 52.622′
48	RT-14	Sadar, Jamalpur	24° 56.170′	89° 55.721′
49	BD-10908	Sharishabari, Jamalpur	24° 45.103′	89° 49.012′
50	BD-10909	Sharishabari, Jamalpur	24° 45.918′	89° 49.108′
51	BD-10910	Sharishabari, Jamalpur	24° 45.440′	89° 49.828′
52	BD-10911	Sharishabari, Jamalpur	24° 45.192′	89° 49.415′
53	BD-10912	Sharishabari, Jamalpur	24° 45.142′	89° 49.914′
54	AM-29	Kazipur, Sirajganj	24° 41.925′	89° 42.978′
55	BD-10899	Galachipa, Patuakhali	22° 14.62′	90° 23.39′
56	BD-10914	Kotalipara, Gopalganj	22° 59.0′	89° 59.30′
57	BD-10926	Charfashion, Bhola	22° 11.283′	90° 47.124′
58	BD-10901	Madarganj, Jamalpur	24° 53.026′	89° 42.296′
59	BD-10902	Madarganj, Jamalpur	24° 53.026′	89° 42.296′
60	BD-10907	Sharishabari, Jamalpur	24° 45.662′	89° 49.828′
61	BD-10939	Khetlal, Joypurhat	25° 1.5′	89° 8′
62	KASI-115	Muksudpur, Gopalganj	23° 19′	89° 52′
63	BD-10940	Sadar, Gazipur	24° 0′	90° 25.30′
64	RI-02	Ramgarh, Khagrachori	22°.59.97′	91° 42.79′
65	RI-12	Ramgarh, Khagrachori	22°.59.58′	90° 41.83′
66	BD-10889	Kalapara, Patuakhali	21°.58.918′	90° 13.60′
67	BD-10890	Amtali, Barguna	22°.05.115′	90° 14.178′
68	AMS-08	Amtali, Barguna	22°.05.115′	90° 14.178′
69	AMS-10	Kalapara, Patuakhali	22°.02.056′	90° 17.005′
70	AMS-21	Galachipa, Patuakhali	22°.10.413′	90° 23.885′
71	AMS-26	Sadar, Patuakhali	22°.16.437′	90° 19.355′
72	AMS-39	Nalsity, Jhalokati	22°.38.203′	90° 20.966′
73	AMS-42	Babuganj, Barisal	22°.46.966′	90° 18.834′
74	AMS-45	Babuganj, Barisal	22°.47.167′	90° 19.888′
75	AHM-46	Babuganj, Barisal	22°.39.03′	90° 00.51′
76	AHM-46(1)	Wajirpur, Barisal	22° 48.42′	90° 14.42′
77	BD-10941	Sadar, Barisal	22°.44.170′	90° 11.124′
78	AHM-142	Jajira, Shariatpur	23°.19.259′	90° 08.421′
79	AHM-143	Jajira, Shariatpur	23°.15.838′	90° 12.381′
80	IA-52	Tongibari, Munshiganj	23°.30.762′	90° 29.715′

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81	BD-10891	Aamtali, Barguna	22° 12.889′	90° 17.853′
82	BD-10893	Galachipa, Patuakhali	22° 10.413′	90° 23.855′
83	AMS-30	Sadar, Patuakhali	22° 21.90′	90° 23.90′
84	AMS-31	Sadar, Patuakhali	22° 21.90′	90° 23.90′
85	AMS-12	Aamtali, Barguna	22° 08.008′	90° 23.831′
86	AMS-32	Dumki, Patuakhali	22° 27.495′	90° 21.298′
87	AMS-33	Bakerganj, Barisal	22° 33.512′	90° 19.893′
88	RT-12	Sadar, Jamalpur	24° 56.167′	89° 55.892′
89	RT-20	Sadar, Jamalpur	24° 50.909′	89° 53.465′
90	RT-22	Sharishabari, Jamalpur	24° 45.312′	89° 49.112′
91	RT-11	Sadar, Jamalpur	24° 56.167′	89° 55.892′
92	RT-13	Sadar, Jamalpur	24° 56.167′	89° 55.892′
93	RT-18	Sadar, Jamalpur	24° 56.909′	89° 53.465′
94	RISA-33	Sadar, Bhola	22° 47.582′	90° 37.837′
95	RM-01	Akkelpur, Joypurhat	25° 01.958′	89° 01.610′
96	KASI-20(1)	Kotalipara, Gopalganj	22° 59.0′	89° 59.30′

amine tetra acetic acid (EDTA) 25 mM, NaCl 300 mM, SDS 1 % and deionized water] at a temperature of 65 °C for about 30 min, extraction by phenol (25): chloroform (24): IAA (1), precipitation with ice-cold and extra pure isopropyl alcohol and purification with absolute ethanol, sodium acetate (3M) and 70 % ethanol chronologically was used. Finally, DNA sample was added in 50 µl of Tris-EDTA (TE) buffer to a 1.5 ml micro centrifuge tube to dissolve. After completely dissolve the DNA pellet, 4 µl RNase @ 10 mg ml^{-1} was added to isolate DNA and incubated for 1.5 hours at 37 °C. Finally, DNA sample was kept in freezer at -20 °C.

2.2 DNA CONCENTRATION MEASUREMENT AND OPTIMIZATION

The occurrence of quality genomic DNA was confirmed on a 1 % agarose gel which was photographed utilizing a photo documentation technique after being visualized under UV light in UV Transilluminator (Uvitec, UK). In this investigation, DNA samples of all cultivars were confirmed to be of good quality. The amount of genomic DNA was quantified through UV spectrophotometer (Spectronic® GENESYS™ 10 Bio) at 260 nm wavelength. Using the spectrophotometer absorbance; the original DNA concentrations were determined according to the following equation:

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

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)

2.3 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 desired amplified product size, and they were used in the present investigation for microsatellite analysis (Table 2).

2.4 STANDARDIZATION OF PCR AND ITS AMPLIFICATION

The PCR was started with 10 µl volumes comprising 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 of the deoxyribonucleotide triphosphate (dNTPs), 10 µM of primer, 0.5 % DMSO (dimethyl sulfoxide) and required amount of deionized water. This reaction was carried out in an oil-free Eppendorf Mastercycler® nexus Gradient thermal cycler. The following

Table 2: List of microsatellite primers used in this study. Ann. T.: Annealing Temperature, Chr. no.: Chromosome number

Sl.	Locus	Primer sequence (5'-3')	Repeat motif	Ann. T.	Chr. no.	Expected Size (bp)	Reference
1	CAMS-336	F: ggtggaaacttgcttggaga R: cccagaaccatccacctact	$(tc)_{16}$	53°C	3	157	(Minamiyama et al., 2006)
2	CAMS-351	F: cgcatgaagcaaatgtacca R: acctgcagtttgttgttgga	$(tg)_3$…$(ag)_{26}$	51°C	4	240	
3	CAMS-405	F: ttcttgggtcccacactttc R: aggttgaaaggagggcaata	$(tc)_{18}$	53°C	8, 11	241	
4	CAMS-460	F: cctttcacttcagcccacat R: accatccgctaagacgagaa	$(tc)_{20}$	54°C	7	215	
5	CAMS-679	F: tttgcatgttttacccattcc R: atgtgaaacacataggtagcactga	$(tat)_{16}$	53°C	1	200	
6	CAMS-864	F:ctgttgtggaagaagaggaca R: gcttctttttcaacctcctcct	$(aga)_{32}$	54°C	7	222	
7	CAMS-072	F: cccgcgaaatcaaggtaat R: aaagctattgctactgggttcg	$(ac)_{13}$	53°C	5	153	
8	CAMS-117	F: ttgtggaggaaacaagcaaa R: cctcagcccaggagacataa	$(tg)_{21}(ta)_3$	52°C	11	223	
9	CAMS-806	F: tgtcacaagtgtcaaggtaggag R: ccccaaaaattttccctcat	$(aga)_{19}$	54°C	10	227	
10	CAMS-844	F: gcaaagaaaaagaaaagcctga R: ctgcaactgctgcttcattc	$(gaa)_6$	53°C	1	223	
11	CAMS-015	F: tcatgttgattatgcttttgttca R: ccatgtattgtatgatacctgagaaa	$(ac)_7$ at$(ac)_8$a $(ta)_7$	53°C	2	112	
12	CAMS-065	F: ccagtctcatccagcagaca R: catatgctgctcctgcattc	$(ac)_{12}$	52°C		213	
13	CAMS-075	F: actaattacacattctgcattttctc R: aggctcgagtaccacgaaga	$(tg)_{10}$	54°C	5	190	
14	CAMS-478	F: gagtgccatgctgattaagga R: cacgactgtcttgcctgaac	(ag)12	52°C	3	248	
15	CAMS-838	F: ccaggatggtgttaagggttt R: gtcgcatcaatgagcatagg	(aga)19	59°C	6	229	
16	CAMS-861	F: gcatgcaagcttagccaac R: tgagattgaagctagaattttgga	(aga)11	52°C	2	183	

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17	CAMS-880	F: gagccaagaaaaaggtggaa R: caactcatcgttcaacaacaca	(gaa)12	53°C	6	237	(Minamiyama et al., 2006)
18	CAMS-236	F: ttgtagtttgcgtaccatttga R: atgaatccagggttccacaa	(ac)14a (ta)10	54°C	2	191	
19	CAMS-885	F: aacgaaaaacaaacccaatca R: ttgaaattgctgaaactctgaa	(gaa)28	53°C	2	248	
20	CAMS-647	F: cggattcggttgagtcgata R: gtgctttggttcggtctttc	(tat)6tg (tta)3… (tat)21	54°C	3	221	
21	CAMS-173	F: caaccgccagtagacaggtt R: gtgcgtgtgcgtgtgtgtat	(cata)7…(ac)4	52°C	4	169	
22	CAMS-163	F: tccatatagcccgtgtgtga R:gcgtgggaatacaatgctaga	(at)7 (gt)14	53°C	5	250	
23	CAMS-826	F: cttgatctcaagaaccagctacaa R: tgtacattgaagacacggaagaa	(gaa)6 ga (gga)9.. (gaa)3 ga (gaa)3	53°C	8	244	
24	CAMS-855	F: aagtgtcaaggaaggggaca R: cctaaccacccccaaaagtt	(agt)14a (gaa)9	54°C	8	243	
25	CAMS-493	F: tcgatgacgaaaaagtgtgaa R: agggcaaaagacccattctt	(ag)6	53°C	8	225	(Mimura et al., 2012)
26	CAMS-454	F: gagcctcttaatgtatctgaaaaca R: aattttggtgaatcgcacct	(ct)3.. (tc)4c (ct)3.. (tc)5.. (tc)5cc (tc)4	54°C	9	243	
27	CAMS-340	F: tttatgcccattcacaaaataa R: ggacgaattcaccgagtgc	(ta)3… (ag)13	53°C	10	250	
28	CAMS-156	F: ccctatgctttcacaactcct R: acgtggttatgacgataggc	(ac)14a (ta)6	54°C	10	181	
29	Hpms 1-1	F: tcaacccaatattaaggtcacttcc R: ccaggcggggattgtagatg	(ca)12 (ta)4	55°C	1	283	(Lee et al., 2005)
30	AF244121	F: tacctcctcgccaatccttctg R: ttgaaagttctttccatgacaacc	(tag)4IP (gtt)3	52°C	1, 3	234	

Continued on the next page

31	Hpms 1-165	F: ggctatttccgacaaaccctcg R: ccattggtgttttcactgttgtg	(ga)13	53°C	4	213	(Lee et al., 2005)
32	Hpms 2-23	F: ccctcggctcaggataaatacc R: ccccagactcccactttgtg	(ttg)7 (gt)9	54°C	5	127	
33	Hpms 1-5	F: ccaaacgaaccgatgaacactc R: gacaatgttgaaaaaggtggaagac	(at)11 (gt)17	53°C	6	311	
34	Hpms AT2-20	F: tgcactgtcttgtgttaaaatgacg R: aaaattgcacaaatatggctgctg	(aat)18	52°C	6	148	
35	Hpms CaSIG19	F: catgaatttcgtcttgaaggtccc R: aagggtgtatcgtacgcagcctta	(ct)6 (at) (gtat)58	54°C	7	218	
36	Hpms 2-21	F: tttttcaattgatgcatgaccgata R: catgtcattttgtcattgatttgg	$(at)_{11}$ $(ac)_{9}$ $(atac)_{10}$	55°C	10	295	
37	Hpms 1-172	F: gggtttgcatgatctaagcatttt R: cgctggaatgcattgtcaaaga	$(ga)_{15}$	58°C	11	344	
38	Hpms 2-2	F: gcaaggatgcttagttgggtgtc R: tcccaaaattaccttgcagcac	$(gt)_{9}$	55°C	11	146	
39	Hpms E-075	F: gcggctcagcagaaagagagag R: tgccacagctggagaacgtaaa	$(acc)_{6}$	52°C	12	205	(Yi et al., 2006)

"touchdown" PCR settings were used to amplify SSRs: 94 °C 3 min^{-1} denaturation, 11 cycles of 94 °C 0.5 min^{-1}, 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; finally, extension for 5 min. The PCR products were resolved electrophoretically on 2 % agarose gel in 1X TBE to check amplification. The PCR procedure was regarded correct when the primer showed decent band, decreased smearing, and amplified the template DNA at target genomic region.

2.5 PCR PRODUCTS SEPARATION AND VISUALIZATION USING ELECTROPHORESIS

The products of PCR were separated on 5 % denatured polyacrylamide gel using acrylamide: bis-acrylamide (19:1), 10 % APS, 10X TBE buffer, and ultrapure Temed. Triple Wide Mini-Vertical Electrophoresis System (Model: MGV-202-33, CBS Scientific, USA) was used to perform the electrophoresis. Upon loading of PCR products, run the gel maintaining 20 ^{0}C temperature

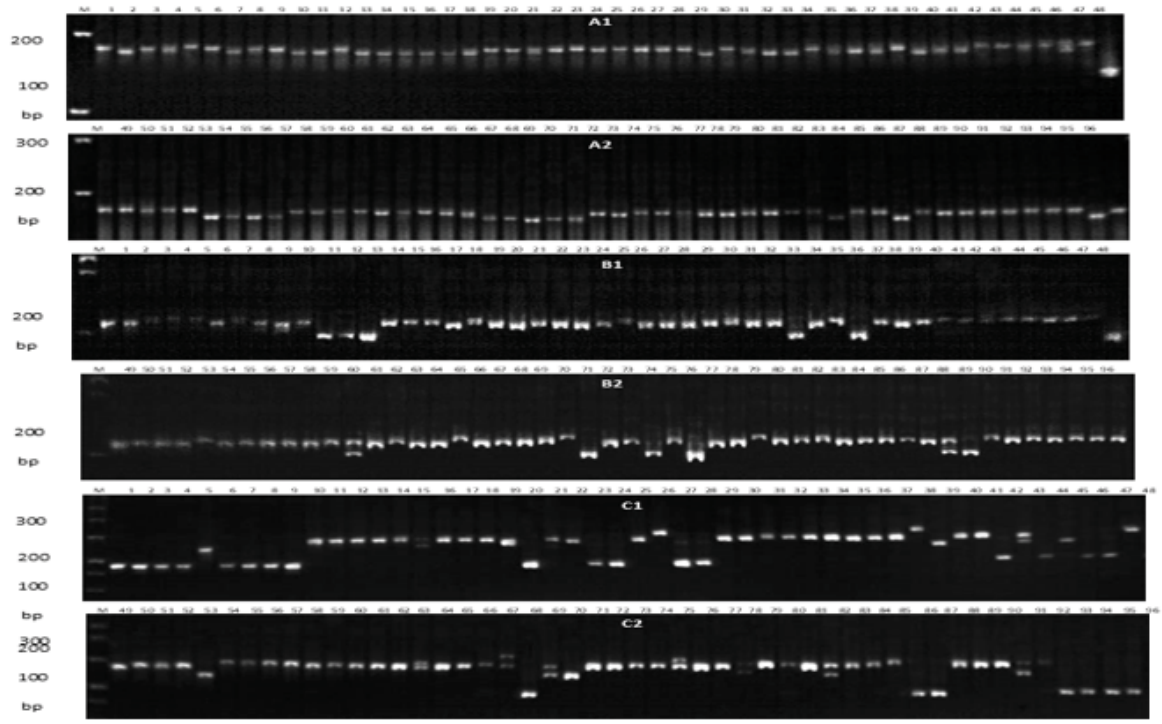

Figure 1: Microsatellite profiles of 96 winter local chilli cultivars at locus CAMS-679 (A1, A2), CAMS-117 (B1, B2) and CAMS-647 (C1, C2); M: Molecular wt. marker (100 bp DNA ladder). Lane 01: BD-10878; Lane 02: BD-10879; Lane 03: BD-10880; Lane 04: BD-10881; Lane 05: BD-10882; Lane 06: BD-10883; Lane 07: BD-10884; Lane 08: BD-10885 Lane 09: BD-10886; Lane 10: BD-10887; Lane 11: BD-10888; Lane 12: BD-10892; Lane 13: BD-10894; Lane 14: BD-10895; Lane 15: BD-10896; Lane 16: BD-10897; Lane 17: BD-10898; Lane 18: BD-10938; Lane 19: BD-10934; Lane 20: BD-10935; Lane 21: BD-10936; Lane 22: BD-10913; Lane 23: KASI-49; Lane 24: BD-10916; Lane 25: BD-10917; Lane 26: BD-10918; Lane 27: BD-10919; Lane 28: BD-10920; Lane 29: RISA-23; Lane 30: BD-10921; Lane 31: BD-10922; Lane 32: BD-10923; Lane 33: BD-10924; Lane 34: BD-10925; Lane 35: BD-10927; Lane 36: BD-10928; Lane 37: BD-10929; Lane 38: BD-10930; Lane 39: BD-10931; Lane 40: BD-10932; Lane 41: BD-10933; Lane 42: BD-10900; Lane 43: BD-10903; Lane44: BD-10903; Lane 45: BD-10905; Lane 46: BD-10906; Lane 47: RT-09; Lane 48: RT-14; Lane 49: BD-10908; Lane 50: BD-10909 Lane 51: BD-10910; Lane 52: BD-10911; Lane 53: BD-10912, Lane 54: AM-29; Lane 55: BD-10899; Lane 56: BD-10914; Lane 57: BD-10926; Lane 58: BD-10901; Lane 59: BD-10902; Lane 60: BD-10907; Lane 61: BD-10939; Lane 62: KASI-115; Lane 63: KASI-115; Lane 64: RI-02; Lane 65: RI-12; Lane 66: BD-10889; Lane 67: BD-10890; Lane 68: AMS-08; Lane 69: AMS-10; Lane 70: AMS-21; Lane 71: AMS-26; Lane 72: AMS-39; Lane 73: AMS-42; Lane 74: AMS-45; Lane 75: AHM-46; Lane 76: AHM-46(1); Lane 77: BD-10941; Lane 78: AHM-142; Lane 79: AHM-143; Lane 80: IA-52; Lane 81: BD-10891; Lane 82: BD-10893; Lane 83: AMS-30; Lane 84: AMS-31; Lane 85: AMS-12; Lane 86: AMS-32; Lane 87: AMS-33; Lane 88: RT-12; Lane 89: RT-20; Lane 90: RT-22; Lane 91: RT-11; Lane 92: RT-13; Lane 93: RT-18; Lane 94: RISA-33; Lane 95: RM-01; Lane 96: KASI-20(1)

at 80-90 V for a set period of time (usually 1 hour for 100 bp) depending on the size of the amplified DNA fragment. Once electrophoresis was completed, the gel was stained with ethidium bromide. For analysis, the individual bands on the glass plate were colored and scored.

2.6 MICROSATELLITE DATA SCORING AND ANALYSIS

Three expert scientists separately assessed the bands representing specific alleles at the microsatellite loci and labelled from the top to the bottom of the gel as A, B & C. Cultivars were hypothetically scored as homozygous (AA, BB, CC) or heterozygous (AB, AC, BC). All loci were combined into a single genotypic data matrix. Allelic frequency estimations were generated to produce statistics of genetic variation (number of observed and effective alleles, Nei's gene diversity, Shannon's information index, heterozygosity, and polymorphism) from genotypic frequency of SSR loci using POPGENE (Version 1.31) (Abouzied et al., 2013). The microsatellite data matrix was deployed to calculate Nei's distance (Nei, 1972), and to produce the corresponding matrix of genetic distance among accessions, while cluster analyses were carried out on the genetic distance matrix by using the UPGMA to determine the relations among accessions (dendrograms) using POPGENE (Version 1.31) (Abouzied et al., 2013). The PIC (polymorphism information content) or gene diversity value of the SSR utilized was computed as PIC= 1- 1- $\Sigma X i^2$; 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 (Islam et al., 2012).

3 RESULTS

3.1 MICROSATELLITE POLYMORPHISM

All 39 microsatellite primers employed in this study were confirmed to be polymorphic based on DNA amplification patterns. Figure 1 illustrates three typical SSR profiles. Table 3 shows the results of the variability parameters analysis for the 39 SSRs in the 96 chilli cultivars. With the 39 SSR loci investigated herein, a total of 123 alleles were found among all chilli cultivars, averaging 3.154 alleles per locus. Variation of allele number ranged from 2 to 8. The locus CAMS-647 yielded the most alleles (8) with sizes ranging from 188 to 279 base pairs. Likewise, 6 alleles (142 to 176 bp and 184 to 240 bp) and 5 alleles (223 to 291 bp) were detected at the loci CAMS-679, CAMS-117 and CAMS-855, respectively, in descending order (Table 3). When all cultivars were considered, the expected heterozygosity (H_E, average 0.484) values for each SSR locus were always higher than the observed heterozygosity (H_O), indicating that the population was homozygous.

PIC values for the 39 primers tested in this work ranged from 0.099 for Hpms 1-165 to 0.806 for CAMS-679, with an average value of 0.484 (Table 3). Among the studied markers CAMS-679, CAMS-855, CAMS-117, CAMS-647, CAMS-236, CAMS-351, CAMS-885, CAMS-340, CAMS-864, CAMS-460, CAMS-844, and CAMS-880 showed higher PIC values (> 0.6) followed by HpmsAT2-20 (0.278), CAMS-015 (0.256), CAMS-156 (0.249), Hpms 1-1 (0.249), CAMS-838 (0.170), Hpms 1-172 (0.170), HpmsE075 (0.117), and Hpms 1-165 (0.099) in descending order. Among the studied markers, allele frequency ranged from 0.281 to 0.948 (Table 3).

Effective allele number was also the highest (5.166) for CAMS-679 following 4.046, 3.912, 3.436, 3.364 and 3.322 for CAMS-855, CAMS-647, CAMS-885, CAMS-236 and CAMS-251, respectively (Table 4). Nei's expected heterozygosity (genetic diversity) ranged from 0.117 (HpmsE075) to 0.811 (CAMS-679) with an average value of 0.484.

The mean Shannon's information index (I) was 0.842, and ranged from 0.205 to 1.713 (Table 4). The highest Shannon's information index (1.713) was recorded in the locus CAMS-647 followed by CAMS-679 (1.617), CAMS-117 (1.589), CAMS-855 (1.444) as against the lowest (0.205) in Hpms 1-165. Ranges of genetic differentiation (Fst) values were 0.834 to 1.000 with an average of 0.927 and gene flow (Nm) values ranged from 0.000 to 0.050 with an average of 0.010 (Table 4).

3.2 NEI'S GENETIC DISTANCE BETWEEN THE CULTIVARS

The genetic distance value (GD) of 4560 (1+2+3+...+95) pairs resulting from a permutation combination of 96 winter chilli cultivars ranged from 0.103 to 0.990 on average. While analyzing 96 cultivars, comparatively higher genetic distance values were observed between the pairs of 55 cultivars, while the pairs of 41 cultivars showed lower GD values (Table 5 and Table 6).

The pair BD-10879 vs RT-22 and BD-10926 vs BD-10920 showed the highest (0.990) genetic distance followed by 0.921 and 0.911 in BD-10887 vs RT-12, BD-10931 vs IA-52, BD-10927 vs BD-10879, RT-11 vs BD-10887, RT-11 vs BD-10926, RT-22 vs BD10931 and IA-52 vs BD-10934, respectively (Table 5). The pair between AMS-30 and BD-10893 showed the lowest (0.103) genetic distance followed by BD-10883 with BD-10880

Table 3: Variability of simple sequence repeat marker used for genetic analysis of chilli cultivars

Locus	No. of allele	Allele sizes (bp)	Major allele frequency	Obs Het (H_o)	Exp Het (H_E)	PIC
CAMS-015	3	100, 106, 110	0.854	0.000	0.258	0.256
CAMS-065	4	197, 209, 215, 239	0.479	0.000	0.578	0.575
CAMS-072	3	153, 166, 173	0.677	0.000	0.476	0.474
CAMS-075	4	178, 194, 207, 218	0.615	0.000	0.554	0.551
CAMS-117	6	184, 193, 208, 222, 227, 240	0.500	0.000	0.633	0.750
CAMS-156	2	176, 185	0.854	0.000	0.250	0.249
CAMS-163	2	136, 148	0.802	0.000	0.319	0.317
CAMS-173	3	146, 159, 170	0.688	0.000	0.459	0.457
CAMS-236	4	182, 198, 199, 202	0.385	0.000	0.706	0.703
CAMS-336	3	152, 173, 183	0.750	0.000	0.401	0.398
CAMS-340	4	245, 260, 272, 287	0.432	0.000	0.662	0.658
CAMS-351	4	179, 189, 200, 220	0.427	0.000	0.703	0.699
CAMS-405	3	207, 226, 244	0.552	0.000	0.574	0.571
CAMS-454	2	221, 240	0.583	0.000	0.489	0.486
CAMS-460	3	195, 209, 218	0.490	0.000	0.633	0.630
CAMS-478	2	215, 230	0.646	0.000	0.460	0.457
CAMS-493	3	201, 213, 225	0.510	0.000	0.571	0.568
CAMS-647	8	188, 198, 206, 220, 235, 239, 256, 279	0.406	0.000	0.748	0.744
CAMS-679	6	142, 147, 154, 160, 168, 176	0.281	0.000	0.811	0.806
CAMS-806	3	209, 222, 233	0.667	0.000	0.476	0.474
CAMS-826	3	215, 229, 258	0.823	0.000	0.307	0.306
CAMS-838	2	160, 164	0.906	0.000	0.171	0.170
CAMS-844	3	198, 210, 219	0.490	0.000	0.633	0.630
CAMS-855	5	223, 239, 252, 270, 291	0.292	0.000	0.757	0.753
CAMS-861	3	209, 230, 240	0.563	0.000	0.569	0.566
CAMS-864	4	205, 231, 264, 291	0.427	0.000	0.649	0.645
CAMS-880	3	205, 219, 231	0.521	0.000	0.615	0.612
CAMS-885	4	200, 209, 216, 224	0.354	0.000	0.713	0.683
Hpms 1-1	2	247, 262	0.854	0.000	0.250	0.249
Hpms 1-5	3	266, 285, 312	0.531	0.000	0.559	0.556
Hpms 1-165	2	191, 202	0.948	0.000	0.099	0.099
Hpms 1-172	2	280, 300	0.906	0.000	0.171	0.170
Hpms 2-2	2	156, 167	0.406	0.000	0.485	0.482
Hpms 2-21	2	273, 294	0.625	0.000	0.471	0.469
Hpms 2-23	2	205, 218	0.740	0.000	0.387	0.385
HpmsAT2-20	2	143, 152	0.833	0.000	0.279	0.278
HpmsCaSIG19	2	209, 220	0.760	0.000	0.366	0.364
HpmsE075	2	208, 220	0.938	0.000	0.118	0.117
AF244121	3	93, 111, 120	0.542	0.000	0.526	0.523
Mean	3.154		0.617	0.000	0.484	0.484

Table 4: Summary of genetic variation statistics for all loci used for 96 winter chilli cultivars analysis

Locus	Observed number of alleles (na)	Effective number of alleles (ne)	Genetic diversity	Shannon's Information Index (I)	Genetic differentiation (Fst)	Gene flow (Nm*)
CAMS-336	3	1.662	0.398	0.703	1.000	0.000
CAMS-351	4	3.322	0.699	1.288	1.000	0.000
CAMS-405	3	2.331	0.571	0.942	1.000	0.000
CAMS-460	3	2.700	0.630	1.046	1.000	0.000
CAMS-679	6	5.166	0.753	1.617	1.000	0.000
CAMS-864	4	2.818	0.645	1.159	1.000	0.000
CAMS-072	3	1.900	0.474	0.802	1.000	0.000
CAMS-117	6	2.703	0.744	1.589	1.000	0.000
CAMS-806	3	1.900	0.474	0.781	0.989	0.003
CAMS-844	3	2.700	0.630	1.046	1.000	0.000
CAMS-015	3	1.345	0.256	0.491	0.838	0.093
CAMS-065	4	2.351	0.675	0.968	0.955	0.005
CAMS-075	4	2.227	0.651	0.972	0.978	0.003
CAMS-478	2	1.843	0.458	0.650	1.000	0.000
CAMS-838	2	1.205	0.170	0.311	0.968	0.004
CAMS-861	3	2.305	0.566	0.937	1.000	0.000
CAMS-880	3	2.577	0.612	1.020	1.000	0.000
CAMS-236	4	3.364	0.703	1.287	1.000	0.000
CAMS-885	4	3.436	0.709	1.292	0.863	0.040
CAMS-647	8	3.912	0.806	1.713	1.000	0.000
CAMS-173	3	1.841	0.457	0.762	0.951	0.004
CAMS-163	2	1.465	0.318	0.498	0.901	0.005
CAMS-826	3	1.441	0.306	0.582	0.891	0.031
CAMS-855	5	4.046	0.734	1.444	0.972	0.007
CAMS-493	3	2.312	0.568	0.916	1.000	0.000
CAMS-454	2	1.946	0.486	0.679	1.000	0.000
CAMS-340	4	2.922	0.658	1.145	0.834	0.050
CAMS-156	2	1.332	0.249	0.415	0.962	0.004
Hpms 1-1	2	1.332	0.249	0.415	0.932	0.007
AF244121	3	2.097	0.523	0.804	0.911	0.008
Hpms 1-165	2	1.110	0.099	0.205	0.874	0.034
Hpms 2-23	2	1.627	0.385	0.574	0.901	0.009
Hpms 1-5	3	2.251	0.556	0.894	0.911	0.008
HpmsAT2-20	2	1.385	0.278	0.451	0.904	0.009
HpmsCaSIG19	2	1.573	0.364	0.551	0.952	0.004
Hpms 2-21	2	1.882	0.469	0.662	0.899	0.023
Hpms 1-172	2	1.205	0.170	0.311	0.879	0.032
Hpms 2-2	2	1.932	0.482	0.676	0.918	0.008
HpmsE075	2	1.133	0.117	0.234	0.895	0.022
Mean	3.154	2.220	0.490	0.842	0.927	0.010

Nm* = Gene flow estimated from Fst = 0.25 (1 - Fst)/Fst, Fst = Genetic differentiation

Table 5: List of genotype pairs of winter chilli showed higher values of Nei's (1972) genetic distance

Genotype pair				Genetic Distance	Genotype pair				Genetic Distance
1	BD-10879	vs	RT-22	0.990	29	RT-20	vs	BD-10896	0.799
2	BD-10926	vs	BD-10920	0.990	30	IA-52	vs	BD-10900	0.799
3	BD-10887	vs	RT-12	0.921	31	BD-10940	vs	BD-10909	0.799
4	BD-10931	vs	IA-52	0.921	32	BD-10931	vs	BD-10913	0.799
5	BD-10927	vs	BD-10879	0.921	33	RT-11	vs	BD-10914	0.799
6	RT-11	vs	BD-10887	0.921	34	RT-22	vs	BD-10916	0.799
7	RT-11	vs	BD-10926	0.921	35	RT-22	vs	BD-10930	0.799
8	RT-22	vs	BD-10931	0.921	36	AHM-46	vs	BD-10906	0.790
9	IA-52	vs	BD-10934	0.911	37	RT-20	vs	BD-10938	0.790
10	BD-10878	vs	BD-10918	0.857	38	BD-10917	vs	BD-10922	0.745
11	BD-10879	vs	RT-20	0.857	39	BD-10918	vs	AM-29	0.745
12	BD-10917	vs	BD-10902	0.857	40	BD-10920	vs	BD-10911	0.745
13	BD-10926	vs	BD-10941	0.857	41	BD-10903	vs	BD-10891	0.745
14	BD-10926	vs	RT-18	0.857	42	IA-52	vs	BD-10925	0.745
15	BD-10931	vs	RT-13	0.857	43	BD-10887	vs	BD-10939	0.745
16	BD-10917	vs	BD-10884	0.857	44	BD-10934	vs	BD-10929	0.736
17	BD-10902	vs	BD-10917	0.857	45	BD-10920	vs	BD-10901	0.695
18	RT-13	vs	BD-10935	0.857	46	BD-10920	vs	BD-10912	0.695
19	BD-10926	vs	AHM-46	0.848	47	BD-10926	vs	RM-01	0.695
20	BD-10878	vs	RI-02	0.799	48	RT-22	vs	BD-10886	0.695
21	BD-10884	vs	RT-11	0.799	49	IA-52	vs	BD-10888	0.695
22	BD-10909	vs	BD-10940	0.799	50	IA-52	vs	BD-10933	0.695
23	BD-10917	vs	BD-10908	0.799	51	BD-10884	vs	BD-10893	0.686
24	BD-10927	vs	IA-52	0.799	52	RT-20	vs	BD-10885	0.648
25	BD-10935	vs	BD-10903	0.799	53	RT-22	vs	BD-10890	0.648
26	BD-10916	vs	BD-10878	0.799	54	BD-10908	vs	BD-10921	0.648
27	BD-10926	vs	BD-10881	0.799	55	BD-10939	vs	BD-10936	0.648
28	BD-10879	vs	BD-10889	0.799					

and BD-10882 (0.122), BD-10923 vs BD-10932 (0.144), RISA-33 vs KASI-20(1) (0.167), BD-10897 vs BD-10898 (0.181) and so on (Table 6).

3.3 PHYLOGENETIC DENDROGRAM

The UPGMA cluster analysis generated a dendrogram that divided 96 chilli cultivars into two main group "A" and "B" where only one cultivar i.e. BD-10917 congregated in a separate group "B" and others (95 cultivars) belong to group "A" (Figure 2). However, Group "A" divided in two sub-group "A1" and "A2". Sub-group "A1" was split into two more sub-group ("A1.a" and "A1.b"), where sub-clusters "A1.a1" gathered eight cultivars (BD-10878, BD-10879, BD-10880, BD-10883, BD-10885, BD-10881, BD-10882 and BD-10884). Sub-cluster "A1.a2" grouped 17 cultivars forming, A1.a2.a3.a5, A1.a2.a3.a6 and A1.a2.a4 sub-clusters contained five (BD-10900, BD-10903, BD-10904, BD-10905, BD-10906), nine (BD-10908, BD-10909, BD-10911, BD-10910, BD-10901, BD-10902, BD-10912, BD-10907 and BD-10939) and three (RT-09, RT-14 and AHM-142) cultivars, respectively (Figure 2 and Table 7).

A total of 29 cultivars were clustered into sub-cluster A1.b, which was further divided into four sub-clusters viz., A1.b1, A1.b2.b3.b5, A1.b2.b3.b6 and A1.b2.b4. Similarly, sub-cluster A2 divided into another two sub-

clusters ("A2.a", "A2.b"), where sub-clusters "A2.a1" assembled seven cultivars (BD-10886, KASI-49, BD-10916, KAI-115, RI-12, BD-10934 and BD-10913) whereas only four cultivars viz., BD-10935, BD-10918, BD-10919 and BD-10920 accumulated in sub-cluster "A2.a2" (Figure 4 and Table 7). However, 30 cultivars separated into seven sub-clusters of A2.b in where maximum five cultivars accumulated in four sub-clusters (A2.b1.b4, A2.b2.b5, A2.b2.b6.b8.b11 and A2.b2.b6.b8.b12), four cultivars gathered in 2 sub-clusters (A2.b2.b6.b7.b9 and A2.b2.b6.b7.b10) and sub-cluster A2.b1.b3 accumulated only two cultivars viz., BD-10929 and AMS-39 (Figure 2 and Table 7).

4 DISCUSSION

Among 50 primers screened, only 39 produced polymorphism and were used for final analysis of 96 winter chilli cultivars on the basis of easily scorable amplified bands (Table 3). All markers were observed to be polymorphic, expressing a total of 123 alleles with an average value of 3.15 alleles per locus in the analysis of 96 winter chilli cultivars. The majority of the primers (25) amplified 3-8 alleles per locus (Table 3), where the highest number of alleles (8) were amplified by the locus CAMS-647. However, Di Dato et al. (2015) observed 10 alleles in *Capsicum annuum* while analyzing with CAMS-647 marker. In another experiment carried out by Dhaliwal et al. (2014) identified the most divergent genotype among the six elite lines of chilli pepper by employing 58 SSR markers. Thirty produced polymorphic bands, revealing a total of 83 alleles with an average of 2.67 alleles per locus. Hossain et al. (2014) evaluated the genetic diversity within 22 chilli germplasm by using four microsatellite markers. All the microsatellite markers were found polymorphic in all studied germplasm. 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). The average numbers of allele (3.15) showed substantial variations compared with those of previous studies might be due to the high number of diverse chilli cultivars used in present study. The observed differences in allelic length for each locus indicated the presence of broad genetic base amongst the chilli cultivars. The wide genetic base might due to the high yield of polymorphic markers as reported by Molla et al. (2015)but there is possible uncertainty of linkage with the important genes. In contrast, there are better possibilities of linkage detection with important genes if SSRs are developed from candidate genes. To the best of our knowledge, there is no such report on SSR markers development from candidate gene sequences in rice. So the present study was aimed to identify and analyse SSRs from salt responsive candidate genes of rice. Results: In the present study, based on the comprehensive literature survey, we selected 220 different salt responsive genes of rice. Out of them, 106 genes were found to contain 180 microsatellite loci with, tri-nucleotide motifs (56%.

The PIC values, the reflection of allele diversity, offer an estimate of the discriminating power of a marker by taking into account not only the number of alleles at a locus, but also relative frequencies of these alleles. The genetic diversity of the cultivars chosen determines the PIC values, and this study featured a large number of traditional varieties, which would increase the PIC values. It is important to point out that the selection by breeders have increased the frequency of the alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them (Cao et al., 1998).

All of the SSRs were found to be polymorphic and useful for defining genotypic variation (i.e., PIC values different from zero) (Table 3). Twelve 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. (2005), Mimura et al. (2012) and Minamiyama et al. (2006). Lower PIC values indicate the presence of closely related cultivars; while higher PIC values indicate the presence of diverse cultivars. The observed high PIC values could be related to the utilization of di-nucleotide repeats as well as genotypic variations as reported by Islam et al. (2018). The present investigation had a high proportion of traditional varieties which would have the effect of increasing the PIC values. It is important to indicate that the selection by the breeders had increased the frequency of alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them (Roychowdhury et al., 2014). The number of alleles amplified by a primer and its PIC values also depends upon the repeat number and the repeat sequence of the microsatellite (Rahman et al., 2010). The results of present investigation are in agreement with those of Minamiyama et al. (2006) who showed that (tat), (tg), (ta) and (gaa) repeats yield higher number of alleles and higher PIC values. CAMS-647, CAMS-679, CAMS-117, CAMS-855, CAMS-885 and CAMS-236 having (tat)n, (tg)n, (gaa)n and (ac)n repeat were the most informative microsatellite markers for this set of cultivars, as they yielded five to eight alleles. For CAMS-647 [$(tat)_6tg(tta)_3$…$(tat)_{21}$], CAMS-117 [$(tg)_{21}(ta)_3$] and CAMS-679 [$(tat)_{16}$], showed eight, six and six alleles and average PIC values 0.744, 0.808, 0.806 and 0.750, respectively in analysis of 96 winter chilli cultivars that were not uncommon in terms of the number of repeats and the repeat motif (Table 2 and Table 3). Indeed, the incredibly beneficial markers are extremely valuable for genetic

Table 6: List of cultivars pairs of winter chilli showed lower values of Nei's (1972) genetic distance

Genotype pair				Genetic Distance	Genotype pair				Genetic Distance
1	AMS-30	vs	BD-10893	0.103	22	RT-09	vs	BD-10910	0.267
2	BD-10883	vs	BD-10880	0.122	23	AMS-21	vs	AMS-08	0.267
3	BD-10883	vs	BD-10882	0.122	24	BD-10895	vs	AMS-10	0.267
4	BD-10932	vs	BD-10923	0.144	25	AMS-21	vs	AMS-39	0.267
5	KASI-20(1)	vs	RISA-33	0.167	26	AHM-143	vs	AHM-142	0.267
6	BD-10898	vs	BD-10897	0.181	27	AMS-32	vs	AMS-31	0.267
7	BD-10894	vs	BD-10892	0.191	28	AHM-46(1)	vs	AMS-45	0.276
8	BD-10892	vs	BD-10895	0.191	29	AHM-143	vs	AHM-46(1)	0.286
9	RT-09	vs	BD-10904	0.191	30	AHM-46(1)	vs	KASI-20(1)	0.286
10	RT-14	vs	RT-09	0.191	31	BD-10924	vs	BD-10932	0.295
11	BD-10892	vs	AMS-26	0.191	32	BD-10910	vs	BD-10907	0.295
12	BD-10895	vs	BD-10894	0.216	33	AMS-39	vs	AMS-42	0.295
13	BD-10892	vs	BD-10928	0.216	34	RISA-33	vs	AHM-143	0.295
14	AMS-26	vs	AMS-21	0.216	35	AMS-33	vs	RT-12	0.295
15	AMS-33	vs	AMS-12	0.216	36	BD-10894	vs	BD-10898	0.314
16	RISA-33	vs	AMS-33	0.216	37	BD-10894	vs	RISA-23	0.323
17	BD-10895	vs	BD-10924	0.241	38	AMS-08	vs	BD-10919	0.353
18	RT-12	vs	KAI-115	0.241	39	AMS-10	vs	BD-10899	0.353
19	BD-10928	vs	AMS-32	0.241	40	AMS-08	vs	RI-12	0.353
20	AMS-31	vs	AMS-30	0.258	41	BD-10880	vs	RT-14	0.416
21	AHM-143	vs	KASI-49	0.267	-	-	-	-	-

investigations and determining the level of variation on a certain marker locus (Minamiyama et al., 2006; Sundaram et al., 2008).

According to Nei (1972), higher level of gene diversity values were observed in loci CAMS-679, CAMS-855, CAMS-647 and CAMS-117 and the lower level of gene diversity value was observed in loci HpmsE075, Hpms 1-172, Hpms 1-165 and Hpms 1-1 in analysis of 96 winter chilli cultivars (Table 4). It was observed that marker which detected the highest/higher number of alleles showed higher gene diversity than those detected lower number of alleles which revealed lowest/lower gene diversity. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity. This result is consistent with previous work done by Chen et al. (2012) and Hossain et al. (2014), who observed that the gene diversity at each SSR locus was significantly correlated with the number of alleles detected, number of repeat motif and with the allele size range. The higher genetic diversity as observed in the current study has also been reported in rice (Rahman et al., 2010), mung bean (Molla et al., 2016) and musk melon (Molla et al., 2017). The current study's findings are similar to those of previous could be owing to higher diversity of cultivars used in this analysis.

Study results also demonstrated higher level of genetic differentiation and low level of gene flow values in 96 chilli cultivars were indicative of diversity among the cultivars due to local origin/cultivars (Table 4). Higher genetic distance between genotype pair indicates that genetically they are diverse compare to lower genetic distance value. Basically, this value is an indication of their genetic dissimilarity. Genotype pair with higher value is more dissimilar than a pair with a lower value. The analysis of molecular data revealed different levels of gene diversity among 96 winter chilli cultivars as determined based on the Nei (1972) genetic distance. According to the results of genetic distance, higher values generated in participation of 55 out of 96 cultivars while rest 41 cultivars yielded lower values (Table 5 and Table 6). Hence, 55 chilli cultivars having higher GD values were selected for further evaluation. From the difference between the highest and the lowest genetic distance values, it was revealed that there was wide variability among

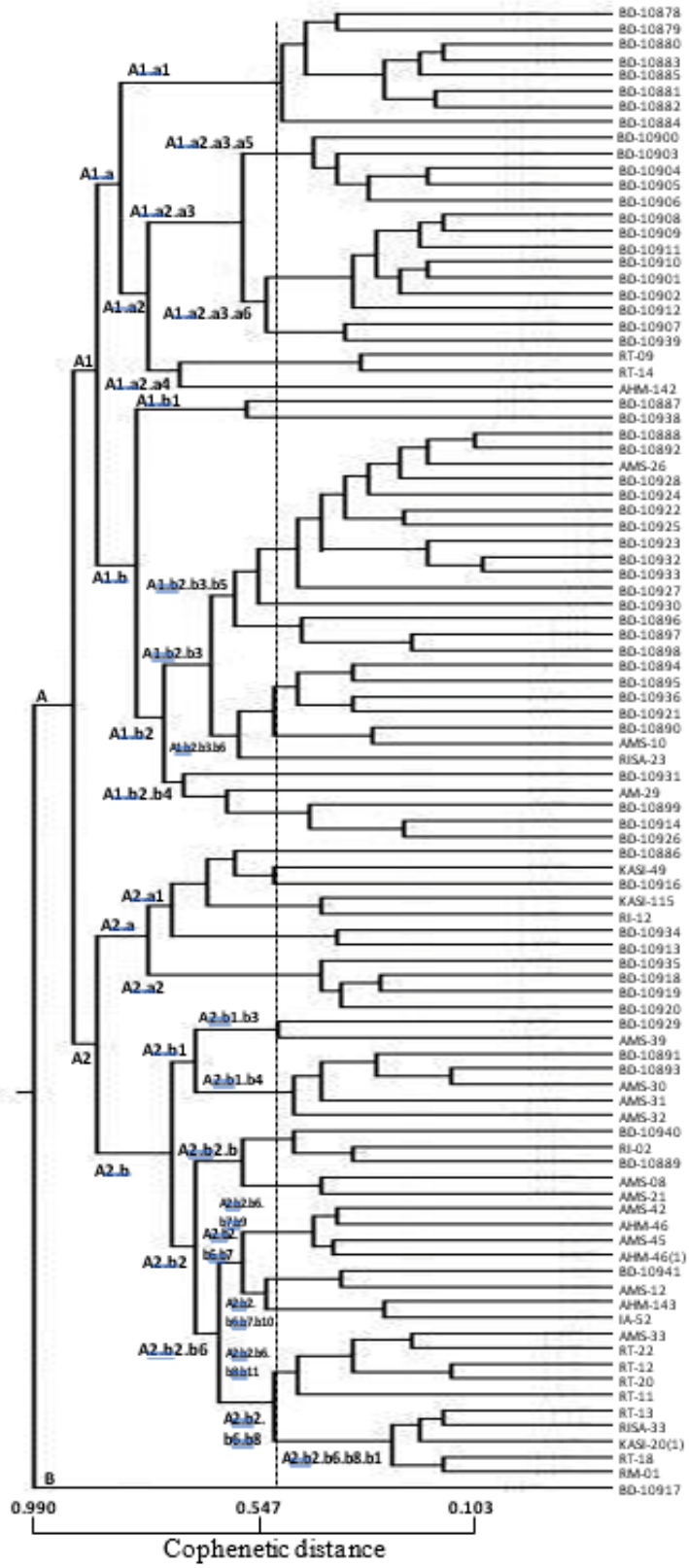

Figure 2: Dendrogram based on Nei's genetic distance, which summarizes the data on the variation between 96 winter local chilli cultivars according to microsatellite analysis

Table 7: Distribution of 96 cultivars according to cluster analysis and selection of diverse cultivars from this cluster

Sl. no.	Genotype	Cluster position	Sl. no.	Selected cultivars	Sl. no.	Genotype	Cluster position	Sl. no.	Selected cultivars
1	BD-10878	A1a1	1	BD-10878	50	BD-10931	A1.b2.b4	28	BD-10931
2	BD-10879		2	BD-10879	51	AM-29		29	AM-29
3	BD-10880		3	BD-10885	52	BD-10899		30	BD-10914
4	BD-10883		4	BD-10881	53	BD-10914		31	BD-10926
5	BD-10885		5	BD-10884	54	BD-10926			
6	BD-10881				55	BD-10886	A2.a1	32	BD-10886
7	BD-10882				56	KASI-49		33	BD-10916
8	BD-10884				57	BD-10916		34	BD-10934
9	BD-10900	A1.a2.a3.a5	6	BD-10900	58	KAI-115		35	BD-10913
10	BD-10903		7	BD-10903	59	RI-12			
11	BD-10904		8	BD-10906	60	BD-10934			
12	BD-10905				61	BD-10913			
					62	BD-10935	A2.a2	36	BD-10935
13	BD-10906				63	BD-10918		37	BD-10918
14	BD-10908	A1.a2.a3.a6	9	BD-10908	64	BD-10919		38	BD-10920
15	BD-10909		10	BD-10909	65	BD-10920			
16	BD-10911		11	BD-10911	66	BD-10929	A2.b1.b3	39	BD-10929
					67	AMS-39			
					68	BD-10891	A2.b1.b4	40	BD-10891
17	BD-10910		12	BD-10901	69	BD-10893		41	BD-10893
18	BD-10901		13	BD-10902	70	AMS-30			
19	BD-10902		14	BD-10912	71	AMS-31			
20	BD-10912		15	BD-10939	72	AMS-32			
21	BD-10907				73	BD-10940	A2.b2.b5	42	BD-10940
22	BD-10939				74	RI-02		43	RI-02
23	RT-09	A1.a2.a4			75	BD-10889		44	BD-10889
24	RT-14				76	AMS-08			
25	AHM-142		-	-	77	AMS-21			
26	BD-10887	A1.b1	16	BD-10887	78	AMS-42	A2.b2.b6.b7.b9	45	AHM-46
27	BD-10938		17	BD-10938	79	AHM-46			
28	BD-10888	A1.b2.b3.b5	18	BD-10888	80	AMS-45			
29	BD-10892		19	BD-10922	81	AHM-46(1)			
30	AMS-26		20	BD-10925	82	BD-10941	A2.b2.b6.b7.b10	46	BD-10941
31	BD-10928		21	BD-10933	83	AMS-12		47	IA-52
32	BD-10924		22	BD-10927	84	AHM-143			
33	BD-10922		23	BD-10930	85	IA-52			

Continued on the next page

34	BD-10925		24	BD-10896	86	AMS-33	A2.b2.b6.b8.b11	48 RT-22
35	BD-10923				87	RT-22		49 RT-12
36	BD-10932				88	RT-12		50 RT-20
37	BD-10933				89	RT-20		51 RT-11
38	BD-10927				90	RT-11		
39	BD-10930				91	RT-13	A2.b2.b6.b8.b12	52 RT-13
40	BD-10896				92	RISA-33		
41	BD-10897				93	KASI-20(1)		
42	BD-10898				94	RT-18		53 RT-18
43	BD-10894	A1.b2.b3.b6	25	BD-10936	95	RM-01		54 RM-01
44	BD-10895		26	BD-10921	96	BD-10917	B	55 BD-10917
45	BD-10936		27	BD-10890				
46	BD-10921							
47	BD-10890							
48	AMS-10							
49	RISA-23							

studied chilli cultivars. 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 may be elucidated 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 to add gene sources from the traditional varieties to HYVs, using genetic fingerprinting and correlating the values with that of the morpho-physiological features to find out the best performing varieties through appropriate breeding programs. 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).

Dendrogram portrayed winter chilli cultivars based on Nei (1972) genetic distance UPGMA cluster analysis broadly placed 96 chilli cultivars into two major groups "A" and "B" in which only one genotype namely BD-10917 congregated in a distinct group "B", and other 95 cultivars clustered in group "A" (Figure 2). The genotype BD-10917 had a distinct status in the dendrogram, because there might have effect of higher genetic distance (Table 5) which might be designated through geographical sources and morphological traits. This genotype was collected from Daulatkhan upazila of Bhola district which is island district of Bangladesh. Moreover, distinct morphological features like hypocotyl color (Purple), stem color before transplanting [Mixture (Green+Purple)], leaf pubescence density (Intermediate), fruit shape (Triangular), blossom end (Sunken and pointed) was observed in this genotype (Molla et al., 2021). Locus CAMS-117 generated 227 bp fragments which was distinguishing band pattern for the cultivars BD-10917, BD-10889 and AHM-46. Among the representation of 96 cultivars, BD-10879 and RT-22 scattered in different sub-cluster (A1a1 and A2.b2.b6.b8.b11) exhibiting the highest genetic distance (0.990) (Table 5 and Figure 2). These two cultivars varied in respect of 14 morphological descriptors in which notable were stem color before transplanting, pedicel position at anthesis, calyx margin shape, anther color, fruit shape at peduncle attachment, fruit shape at blossom end (Molla et al., 2021). Moreover, two cultivars, BD-10879 collected from Galachipa, Patuakhali, and RT-22 collected from Sharishabari, Jamalpur (Table 1) are two widely distanced locations of Bangladesh. However, BD-10880 and BD-10883 were grouped together in same sub-cluster (A1a1) and those cultivars showed similar states in respect of 19 morphological traits such as stem color before and after transplanting, leaf shape, leaf color, pigmentation at node, calyx margin shape, filament color, fruit shape at peduncle attachment, fruit shape, fruit shape at blossom end were remarkable (Molla et al., 2021). In addition, similar geographical sources viz. Sirajganj was observed in case of both cultivars (Table 1). 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 cultivars in different sub-clusters where such values depend on their morphological characters as got selected in different geographical locations.

5 CONCLUSIONS

From this study, it can be concluded that a comparative assessment of the reproducibility of molecular markers has been made for determination of genetic variability among winter growing chilli cultivars in Bangladesh. Higher genetic variability within populations and significant genetic differentiation between populations indicate rich genetic resources of a species. The study also indicated that 55 cultivars derived from different origin were identified as diversified and could be utilized in breeding program for traits of interest. SSR markers have proved to be powerful tools for molecular genetic analysis of chilli cultivars for plant breeding program to assess genetic diversity available. This would allow for the development of new varieties aiming at the improvement of crop productivity withstanding biotic and abiotic stresses.

6 ACKNOWLEDGEMENTS

For the financial support provided in this research, the Secretariat of AFACI, RDA, Korean Republic, has greatly been recognized by the AFACI PAN-ASIAN Project for "Rice, Chili, Cucumber and Melon Collection, Characterization and Promotion in Bangladesh."

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