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Assessment of Genetic Diversity based on Agro-morphological Traits and Genic Microsatellite Markers in Wheat Cultivars

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ABSTRACT: A set of seventeen wheat cultivars were subjected to genetic diversity analysis based on 11 morphometric traits and 20 simple sequence repeat (SSR) markers. The highest polymorphism (93.20%) depicted that the population under study is genetically diverse. Based on polymorphism exhibited by SSR markers, dendrogram was constructed using Jaccard's similarity coefficient using UPGMA method of NTSYS-PC package (version 2.02); the genotypes were grouped into three major clusters with a genetic similarity in the range of 37-67 per cent. Jaccard's similarity coefficient indices were developed on the basis of the scorable banding patterns of the 17 wheat varieties using polymorphic markers. The Jaccard's similarity coefficient values were used as input data to construct a dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and SAHN clustering using the computer package NTSYSpc version 2.0. Using SSR markers, first principal component explained 37.25 per cent of the total variation followed by second component explained 93 per cent variation, third component explained 83 per cent variation, fourth component explained 6.85 per cent variation and the fifth one explained 6.01 per cent variation of the data. In principal component analysis, the genotypes from the HPW and HS series were more distant, indicating that higher genetic diversity resided in them. Results of present study will be useful in understanding the wheat genetic structure and selecting diverse accessions from analysed varieties, to be exploited in a sustainable manner in future breeding programmes.

Keywords: Wheat, Simple Sequence Repeat (SSR), Genetic structure, Diversity

Wheat (*Triticum aestivum* L.) (2n = 6x = 42) belongs to family Poaceae, the most diverse and important family of the plant kingdom and originated in the Middle East. It produces large edible grains and provides about one-half of humans' food calories and a large part of their nutrient requirements. Wheat (*Triticum* spp.) is a worldwide cultivated and domesticated grass. It has been described as the 'King of Cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the International food grain trade.

Wheat has always been subjected to extensive and ceaseless research so as to maximize grain production but also to improve grain yield per unit area. However, there is still considerable room for improvement, especially to amplify efforts for continued genetic improvement of wheat to meet the growing requirements of an ever increasing population. Genetic manipulation is the best way to boost up wheat production. Therefore, it is necessary to estimate and study the genetic variation and mode of inheritance in different plant parameters to initiate productive wheat breeding programs.

The genetic diversity erosion of wheat has been increasingly severe. The narrow genetic diversity leads to vulnerability of biotic stresses and abiotic stresses. Genetic diversity is the ultimate basis for genetic improvement. The knowledge of genetic diversity is critical for their utilization in the improvement of crops. Therefore, it is necessary to investigate the genetic diversity in wheat cultivars, to broaden genetic variation in wheat breeding. Higher extent of genetic diversity represents the wider range of traits related to a species, therefore, genetic diversity in crops is very much helpful and important for global environment and food security [1]. Information on previously recorded plant trait variation is used by plant breeders to plan the future experimentations for producing improved varieties. Earlier for germplasm management, morphological traits were used by the plant breeders to assess the diversity, but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences [2]. But the knowledge of the phenotype given by the morphological and agronomical descriptors is still important. Thus, information of genetic diversity is an essential component in crop improvement. Genetic diversity of a crop or species can be studied based on pedigree data, morphological traits and molecular markers. But, due to higher advantages of molecular markers, these are favoured to detect diversity with more accuracy. Moreover, combinations of different methods are also preferred to capture important traits diversity.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. In recent times, Simple sequence repeats (SSR) marker has been preferred to DNA based marker system due to high levels of polymorphism and information content, selective neutrality, high reproducibility, and wide dispersion in diverse genomes [3, 4]. Simple sequence repeat (SSR) markers has become now one of the most widely used markers in the related researches in crops, especially in the molecular characterization of genetic resources, e.g., soybean [5], rice [6], and wheat [7]. Wider applicability of these markers has been proved by various workers who successfully utilized these markers across the species and genera [8, 9], in wild relatives [10] and in a seed bank collection of improved wheat germplasm [11, 12].

Information generated by using molecular markers, in conjugation with the available morphological description, would not only facilitate greater understanding of the genetic diversity in the available genetic resources, but will also provide reliable means of intellectual property protection. This study was conducted to estimate the genetic divergence among seventeen wheat genotypes with the help of SSR markers. The present study addressed the utilization of microsatellite markers, to determine genetic diversity and relationships at the molecular level among 17 genotypes of wheat to help in the selection of parents to develop high-yielding varieties in breeding programmes.

MATERIALS AND METHODS

Seed Material

Seventeen cultivars of wheat were used for the present study. Out of these cultivars 10 from CSK HPKV, Palampur, 3 cultivars from ICAR, Shimla and rest 4 cultivars were procured from VPKAS, Almora and these cultivars have been recommended for commercial cultivation in Northern Hill and Plain Zones of Himachal Pradesh State. Nucleus seed of these varieties was used for studies and was obtained from Wheat Breeder and Seed Production Scientists of the University. The field experiment was conducted at experimental farm of Department of Seed Science & Technology, CSKHPKV Palampur, during *rabi* season of 2013 and laboratory studies were carried out in the laboratory of Department of Seed Science & Technology, CSKHPKV Palampur. The Experimental farm is situated at $32^0$6' N latitude, $76^0$3' E longitude at an elevation of 1290.8 m (amsl). The soil is acidic in nature with pH ranging from 5.0 to 5.6 and soil texture is silty clay loam. The field experiment was laid out in a RBD with three replications in a block size of 3.0 × 1.5 m^2 with inter and intra row spacing 30 cm and 10 cm, respectively. The detailed list of varieties and their source are mentioned below (Table 1).

Table 1. Wheat cultivars and their source

Sr. No.	Varieties	Source	Year of Release
1.	HPW-42	CSKHPKV, Palampur	1992
2.	HPW-89	CSKHPKV, Palampur	1996
3.	HPW-147	CSKHPKV, Palampur	1999
4.	HPW-155	CSKHPKV, Palampur	2005
5.	HPW-184	CSKHPKV, Palampur	2003
6.	HPW-211	CSKHPKV, Palampur	2006
7.	HPW-236	CSKHPKV, Palampur	2010
8.	HPW-249	CSKHPKV, Palampur	2008
9.	HPW-251	CSKHPKV, Palampur	2007
10.	HPW-349	CSKHPKV, Palampur	2012
11.	HS-295	ICAR, Shimla	1994
12.	HS-490	ICAR, Shimla	2007
13.	HS-507	ICAR, Shimla	2010
14.	VL-616	VPKAS, Almora	1988
15.	VL-829	VPKAS, Almora	2002
16.	VL-892	VPKAS, Almora	2007
17.	VL-907	VPKAS, Almora	2010

Morpho-agronomic Traits Data Recording and Analysis

Observations on morphological characters were recorded on ten randomly selected plants. Each characters was categorized with the help of descriptors provided in the guidelines for the conduct of test for Distinctiveness, Uniformity and Stability (DUS) on bread wheat by Protection of Plant Varieties and Farmer's Right Authority (PPV & FRA), GOI, New Delhi [13] and some characters were categorized as per categorization done by [14]. The data was recorded for various qualitative and quantitative traits *viz.,* Plant growth habit, plant height excluding awns and scurs, plant flag leaf attitude, plant foliage colour, flag leaf anthocyanin coloration of auricle, flag leaf hair on auricle, flag leaf length (cm), flag leaf width (cm), flag leaf waxiness of sheath, flag leaf waxiness of blade, days to ear emergence, ear waxiness, ear length excluding awns and scurs (cm), ear colour, ear shape, ear density, awns presence, awn length (cm), awn colour, awn attitude, peduncle waxiness, peduncle length (cm), peduncle attitude, outer glume pubescence, lower glume shaller width, lower glume shaller shape, lower glume beak shape, lower glume beak length, scurs presence, total number of tillers per plant, days to 75% maturity, number of spikelets per spike, number of seeds per spike, seed yield (qt./ha), biological yield (qt./ha), harvest index (%), seed colour, seed shape, seed size, seed hardness, seed germ width, seed crease, brush hair length, anthocyanin coloration of coleoptiles, germination percentage (%), seedling length (cm), seedling vigour index, seedling dry weight (g), grain coloration with phenol and sodium hydroxide test (NaOH). The Analysis of variance was carried out for each of the characters studied [15]. For computation of genotypic and phenotypic coefficient of variations (GCV & PCV) [16] and for heritability and the genetic advance in per cent of mean [17] standard methods were followed. The genetic divergence of wheat cultivars was estimated using Mahalanobis D^2-statistics [18].

DNA Extraction and SSR Genotyping

Young leaves of each accession were used for DNA extraction following the CTAB method [19] with some modifications. DNA Stocks were prepared in TE buffer and quantification of DNA was done on 0.8% agarose gel by comparing with lambda DNA (Fermentas, Lithuania). We utilized 26 SSR primers from the SSRs developed in wheat. PCR reactions were performed in a10 µL reaction volume as per [20]. The total PCR reaction volume contained 20 ng of template DNA, 15 ng of each primer, 200 µM of each dNTP, 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$, 0.01% gelatin and 0.5U of Taq DNA polymerase. PCR amplification was carried out in Veriti Thermal Cycler (Applied Biosystems, CA, USA) and all PCR reactions were performed at one cycle of 4 minute at 94°C as initial denaturation, followed by 35 cycles with a denaturation step at 94°C for 30 seconds, an annealing step for 45 seconds at respective annealing temperature of each primer in a range of 47°C-53.5°C (Table 2) and an extension step at 72°C for 1 minute, followed by last cycle of extension at 72°C for 7 min. PCR products were first checked on 3% agarose gel and then resolved in 6% polyacrylamide gel at a constant 65 Watt at room temperature for 90 minutes. Gels were prepared and run in 1X TBE buffer and visualization of fragments was done using silver-staining. Sizing of alleles was done with the help of 50 bp DNA ladder (Fermentas, Lithuania).

SSR Data Analysis

All fragments were scored manually as per standard method of SSR scoring and each allele is denoted by an alphabet. The polymorphism information content (PIC) of each marker was calculated according to following formula given by [21] and implemented in CERVUS version 3.0:

n

PICi= 1-∑P2ij

j=i

Where, P_{ij} is the frequency of the j^{th} allele in clone for marker i and summation extends over n patterns. Various genetic diversity estimates such as expected heterozygosity (He), observed heterozygosity (Ho), Shannon information index (I) etc. were calculated with the help of POPGENE version 1.32 [22]. Dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of NTSYSpc 2.0 [23]. Neighbor-Joining tree was constructed using Dice coefficient with the help of DARwin [24]. Bootstrapping with 1000 replicates was also performed with DARwin. Bayesian model based clustering method implemented in STRUCTURE software, version: 2.3.3 [25, 26] was utilized to assess the genetic structure at population level as well as to detect genetic stocks contributing to this germplasm collection. Ancestry model with admixture and correlated allele frequency model was set to get the estimates of posterior probability of data. Ten independent runs were given setting the value of K from 1 to 15 with three iterations for each value of K. Length of burn-in period was set at 100,000 and number of Markov Chain Monte Carlo (MCMC) repeats after burn-in were set at 100,000. Evanno's method [27] based programme STRUCTURE HARVESTER developed by [28] was used to determine the value of estimated Ln probability of data- LnP (K) and to get the

Table 2. List of *Triticum aestivum L.* microsatellite primer sequences used in the present study

S. No.	Primers	Forward sequence	Reverse sequence	Annealing temperature (Tm)(^{0}C)
1	*Wms 186*	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	53.0^{0}C
2	*Wms 18*	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	53.0^{0}C
3	*Wms 190*	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	53.0^{0}C
4	*Wms 5*	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	53.0^{0}C
5	*Wms 111*	TCTGTAGGCTCTCTCCGACTG	ACCTGATCAGATCCCACTCG	53.0^{0}C
6	*Wms 391*	ATAGCGAAGTCTCCCTACTCC	ATGTGCATGTCGGACGC	53.0^{0}C
7	*Wmc 11*	TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	53.5^{0}C
8	*Wms 3*	GCAGCACTGGTACATTT	AATATCGCATCACTATCCCA	53.5^{0}C
9	*Wms 410*	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	53.5^{0}C
10	*Wms 155*	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	50.0^{0}C
11	*Wms 408*	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	50.0^{0}C
12	*Wms 249*	CAAATGGATCGAGAAAGGGA	CTGCCATTTTTCTGCATCTAC	50.0^{0}C
13	*Wms 437*	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTTAGCTTA	47.0^{0}C
14	*Xbarc 314*	CTGTGGAAACCAATAAAAACAA	GTGCGCGAATAACTACAAGAAA	47.5^{0}C
15	*Wms 443*	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	50.0^{0}C
16	*Wms 95*	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	50.5^{0}C
17	*Wms 114*	ACAAACAGAAAATCAAAACCCG	ATCCATCGCCATTGGAGTG	50.5^{0}C
18	*Wmc 94*	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA	47.5^{0}C
19	*Wms 44*	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG	53.0^{0}C
20	*Wms 112*	CTAAACACGACAGCGGTGG	GATATGGCAGCGGTCAG	53.0^{0}C
21	*Xbarc 111*	TAG ACATGTGATGTGCGCTCATT	GCGTATCCCATTGCTCTTCTTCACTAAC	53.5^{0}C
22	*Xcfd 2*	GCGGTCACCAGTAGTTCAACA	CATCTATTGCCAAAATCGCA	50.5^{0}C
23	*Xcfa 2193*	GGTTGCAGTTTCCACCTTGT	TCCTCAGAACCCCATTCTTG	51.5^{0}C
24	*Xbarc1046*	GCGGAAGTCCAAAATTAGTATAGG	ACTCCAATGGCAAATACTCAACA	51.5^{0}C
25	*Wmc 506*	CACTTCCTCAACATGCCAGA	CTTTCAATGTGGAAGGCGAC	51.5^{0}C
26	*Wmc 256*	CCAAATCTTCGAACAAGAACCC	ACCGATCGATGGTGTATACTGA	51.5^{0}C

best fit value of K for the data. STRUCTURE was run for all genotypes collectively. Genetic relationships among the genotypes were also analyzed by principal coordinate analysis (PCA) using the Genalex 6.4 program. All the genotypes were plotted on the first two principal axes.

RESULTS AND DISCUSSION

Often, morphological traits are known to be influenced by the environmental factors but the importance of these traits can’t be neglected for analyzing diversity of a crop species as they are the primary constituents of overall diversity that can be detected at first during a diversity study. Many earlier researchers have utilized morphological traits in combination with molecular markers to recover more precision in characteristic variability inferences in many crops including pea [29-31]. Therefore, we conducted morphological traits study together with SSR markers to draw more concrete conclusions.

The analysis of genetic diversity collected from diverse geographical regions is important for deciphering nature and magnitude of variability, heritability, genetic advance, genetic correction between different traits and contribution of various traits towards genetic diversity. Phenotypic characterization coupled with molecular dissection of genetic diversity using SSR markers helps in detecting the occurrence of diversity within population and spelt out precise information on the nature and magnitude of genetic divergence among gene pools for reliable scoring during selection of potential parents for hybridization [32, 33].

Mean, Range, Variance, Co-efficient of Variance, Heritability and Genetic Advance

The statistical analysis of data on quantitative traits showed wide range of variability among the accessions

(Table 3). days to ear emergence, flag leaf length, tillers per plant, awn length, peduncle length, plant length, days to 75 per cent maturity, seed yield, 1000-seed weight, biological yield, spikelets per spike, seeds per spike, seedling dry weight, seedling vigour index except harvest index, germination percentage and seedling length exhibiting thereby the presence of considerable amount of genetic variability under study (Table 3). Earlier workers [34, 35] have also reported significant differences in the genetic material of wheat. Variation among wheat populations was also reported [36] for the characters *viz.*, number of tillers, flag leaf length, flag leaf width and days to heading etc. which are in conformation with the study. The mean numbers of days to ear emergence were 128.02, but it ranged from 122.76 for cultivar HPW211 to 140.33 for HPW251. The average flag leaf length was 24.61 cm with lowest at 18.48 cm for HPW184 to 31.39 cm for HPW249. Rawashdeh *et al.* [34] also characterized 112 wild wheat (*Triticum aegilops* L.) genotypes and 12 population of cultivated wheat (*Triticum aestivum* L.) on the basis of flag leaf length. The number of tillers/plant were highest 12.33 for HPW184 and as low as 7.67 for HS295. Flag leaf width ranged from 1.68 for VL892 to 2.19cm for HPW147. Genetic variation of 96 durum wheat land races was evaluated [37] and cultivars using morphological characters *viz.*, days to heading, flag leaf waxiness of blade, flag leaf length, flag leaf width, spikelet per spike, test weight, plant height, peduncle waxiness, peduncle attitude, peduncle length and spike length. Ear length average was 11.52cm and ranged from 9.02cm for HPW184 to 13.29cm for HPW251. Awn length varied from 4.78cm for cultivar HS507 to 8.79cm for HPW236. Similarly, on the basis of awn length the characterization of wheat × *tritordium* F_1 hybrids and their parents was done [38]. Peduncle length was longest (13.07cm) for HPW155 and shortest (7.59cm) for VL892 with an average value of 10.09cm. The average plant length (cm) was 101.19 with lowest at 85.61 for HPW24 to highest 115.93 for VL616. HPW251 took longest time of 196.33 days for 75% maturity as compared VL892, which matures in 179.67 days. The average 1000-seed weight for all accessions was 46.73g but it was highest for VL616 (51.95g) compared to HPW42 (39.59g). The seed yield

Table 3. Estimates of parameters of variability for various traits in wheat genotypes

Traits	Mean + SEm(±)	Range	PCV (%)	GCV (%)	ECV (%)	h^2bs (%)	GA (as % of mean)
Days to ear emergence	128.02+0.50	122.67-140.33	3.88	3.81	0.70	96.71	7.72
Flag leaf length (cm)	24.613+1.11	18.48-31.39	15.66	13.42	8.07	73.41	23.68
Flag leaf width (cm)	1.85+0.08	1.68-2.19	9.63	5.88	7.63	37.22	7.39
Ear length (cm)	11.52+0.21	9.02-13.29	10.09	9.54	3.27	89.50	18.60
Tillers per plant	9.04+0.45	7.67-12.33	15.22	12.31	8.94	65.48	20.53
Awn length (cm)	6.80+0.08	4.78-8.79	16.97	16.83	2.16	98.38	34.39
Peduncle length (cm)	10.09+0.82	7.59-13.07	18.39	11.21	14.58	37.16	14.08
Plant length (cm)	101.19+1.81	85.61-115.93	7.83	7.15	3.19	83.41	13.46
Days to full maturity	184.35+0.85	179.67-196.33	2.17	2.01	0.82	85.75	3.84
1000 seed weight (g)	46.73+1.07	39.59-51.95	7.08	5.77	4.10	66.48	9.70
Biological yield (kg/plot)	3.91+0.18	2.96-4.85	16.95	15.00	7.89	0.78	1.07
Biological yield (q/ha)	86.92+3.84	65.85-107.84	16.95	15.00	7.89	78.34	27.35
Harvest index (%)	32.66+1.03	31.12-34.23	5.39	1.65	5.64	-9.31	- 1.03
Spikelets per spike	19.33+0.78	16.67-21.67	9.31	5.85	7.24	39.44	7.56
Seeds per spike	61.29+1.36	54.33-65.33	6.30	4.90	3.96	60.60	7.87
Germination percentage (%)	90.76+2.17	86.67-94.67	4.46	1.30	4.26	8.54	0.78
Seedling length (cm)	22.68+1.32	18.90-25.10	11.57	5.11	10.38	19.54	4.65
Seedling dry weight (g)	0.67+0.02	0.54-0.75	9.73	7.40	6.32	57.76	11.58
Seedling vigour index	2073.58+129.80	1746.57-2341.13	13.06	6.75	11.18	26.72	7.19
Seed yield (kg/plot)	1.09+0.05	0.73-1.35	19.95	18.24	8.08	0.84	34.36
Seed yield (q/ha)	24.28+19.96	16.22-30.00	19.96	18.25	8.08	83.59	34.36

S.E.: standard error**, PCV:** phenotypic coefficient of variation**, GCV:** genotypic coefficient of variation**, ECV:** environmental coefficient of variation**, h^2bs:** heritability due to broad sense**, GA:** genetic advance (kg/plot) ranged from 0.73 to 1.35 for HPW42 and HS490, respectively. The seed yield (q/ha) ranged from 16.22 for HPW42 to 29.11 for HS490, respectively. The biological yield (kg/plot) ranged from 65.85 to 107.84 for HPW42 and HS490, respectively. The biological yield (q/ha) ranged from 16.22 for HPW42 to 29.11 for HS490, respectively. HPW147 has maximum harvest index (34.23) as compared to HPW349, which has minimum (31.12). The average numbers of spikelets per spike were 19.33, while range was from 16.67 to 21.67 for HPW42 and HPW236, respectively. Seed per spike were highest for HPW236 (65.33), while minimum for HPW42 (54.33). Germination percentage was ranged from 86.67 to 94.67 for HS507 and HS490 respectively. Seedling length (cm) ranged from 18.90 to 25.10 for Vl616 and VL829 with an average of 22.68. Seedling dry weight (g) was highest (0.75) for HPW236 and lowest (0.54) for HPW251 with an average value of 0.67. The seedling vigour index varied from a range of 1746.57 (lowest) for HS407 to 2341.13 (highest) for cultivar VL907 with an overall average of 2073.58.

The genotypic and phenotypic variances are of little meaning as these do not have any clear limits or ceilings. At the same time, the categorization of the genotypic variance as low or high is difficult, rendering thereby such parameters unsuitable for precise comparison of two populations when expressed in absolute values. To overcome this difficulty, the genotypic and phenotypic coefficient of variation, which are free from the units of measurement, can be conveniently employed for making comparison between populations and different metric traits.

The high PCV and GCV values were recorded for grain yield and tillers per plant, which coincides with earlier findings [39, 40]. The high variability values for seed yield among the genotypes suggest that there is wide scope for selection of high yielding superior genotypes. The knowledge of heritability influences the choice of breeding procedures, and the characters exhibiting high heritability should be given top priority during selection breeding in wheat [41]. Heritability in broad sense was high (>80%) for seed yield, days to ear emergence, ear length, awn length, days to 75 per cent maturity and plant length. Moderate (50-80%) was observed for biological yield, flag leaf length, 1000 seed weight, tillers per plot, seeds per spike and seedling dry weight whereas it was low (<50%) for spikelets per spike, flag leaf width, peduncle length, seedling vigour index, seedling dry weight and harvest index. Similar results of high heritability for various traits were reported by different workers [42] for plant height, grains per spike, 100 grain-weight, harvest index and grain yield; [39] for grain yield per plot and days to heading; [40] also observed high heritability estimates for seed yield per plant (g) and number of tillers per plant. The traits which revealed high heritability revealed lesser influence of the environment and greater role of genetic component of variation. Therefore, selection for these traits on the basis of phenotypic expression would be more effective and can be relied upon.

The estimates of heritability alone fail to indicate the response to selection. The heritability estimates appear to be more meaningful when accompanied by estimates of genetic advance then alone [17]. Thus, the genetic advance has an added edge over heritability as a guiding factor to breeders in various selection programmes. High heritability coupled with high genetic advance was obtained with plant height, grains per spike, 100-grain weight, harvest index and grain yield [42]. High genetic coefficient of variation along with high heritability and genetic advance in grain yield per plot and days to heading was also recorded earlier [39].

SSR Polymorphism and Diversity Analysis

Out of 26 SSRs, 20 primers showed polymorphism and six were monomorphic (Plate1). Twenty SSR primer pairs used in this study amplified 103 alleles with an average of 5.15 alleles per locus. Size of alleles varied from 35 to 500 bp. The highest polymorphism (93.20%) depicted that the population under study is genetically diverse. The Polymorphic information content (PIC), a parameter associated with the discriminating power of markers, ranged from 0.21 (*Wms190*) to 0.50 (*Wms391*) with a

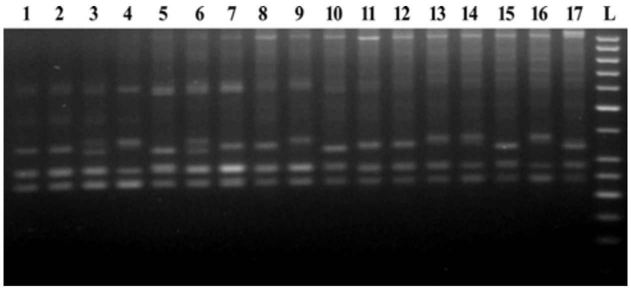

Plate 1. SSR profile in wheat genotypes using primer Wms410, L=50 bp DNA ladder (1, HPW155; 2, HPW249; 3, HPW349; 4, HPW89; 5, HPW211; 6, VL829; 7,VL907; 8, HS507; 9,VL892; 10,HPW42; 11, HPW251; 12,HPW147; 13,VL616; 14, HS490; 15, HPW184; 16, HPW236; 17,HS295)

mean of 0.41 per primer. This could be because the present investigation involved a high proportion of closely related cultivars, resulting in the lower PIC values. Utilization of hypervariable/hyperpolymorphic SSR markers could have contributed to the enhancement of PIC values [43]. High value of MI, which is considered to be over all measure of efficiency to detect polymorphism for markers, is derived from its high polymorphism, EMR and PIC values. All these diversity indices are shown in table 4. High values of all the measures of diversity indicated allelic richness in the analysed cultivars which can be utilized in breeding programmes to get desired plant types for commercial cultivation. Thus, nature of pollination has a remarkable effect on distribution, diversity and richness of alleles within and among populations of a given species. Low level of genetic diversity was observed in peach was thought to be due to its self-pollinating nature [44]. Utility of a set of 20 wheat SSR markers to detect DNA polymorphism was examined [45], identify genotypes and observed that the range of alleles per locus was 1-13, averaging 7.4 and the PIC range was 0.21-0.90, averaging 0.71, also found a similar PIC range (0.21-0.93) and the average PIC for these markers was estimated to be 0.52.

Bayesian Genetic Structure

Detailed knowledge of genetic relatedness among individuals in an association panel is a key factor to avoid spurious associations. Population structure (Q matrix), estimated using STRUCTURE [46] and expressed as membership probabilities, is one way to correct spurious associations due to genetic relatedness. It is often difficult to estimate the true number of population (k). Generally, k is taken to be the value with the highest estimated LnP (D) value returned by STRUCTURE. However, in real situations, few data sets confirm precisely to the STRUCTURE model, and the LnP (D) value keeps increasing when k reaches the true number; Dk, the second-order rate of change of the likelihood function with respect to k performed well in indicating the real number. In this study, the Dk values indicated splitting the maize genotypes into four groups was the most biologically meaningful population structure because of the rapid rate of change in LnP (D) values between successive k. The population structure in the panel

Table 4. Number of scorable and polymorphic SSR bands along with their fragment size generated by 20 primers

S.No.	Primers	n_a	Number of polymorphic fragments	Polymorphic Bands (%)	PIC Value	EMR	MI	Fragments Size (bp)
1	*Wms 186*	6	6	100.00	0.37	6	2.22	50-150
2	*Wms 18*	5	5	100.00	0.28	5	1.40	50-380
3	*Wms 190*	7	7	100.00	0.21	7	1.47	80-500
4	*Wms 5*	7	7	100.00	0.46	7	3.22	40-300
5	*Wms 111*	6	6	100.00	0.48	6	2.88	80-220
6	*Wms 391*	4	4	100.00	0.50	4	2.00	40-170
7	*Wmc 11*	4	4	100.00	0.49	4	1.96	150-250
8	*Wms 3*	4	4	100.00	0.46	4	1.84	35-90
9	*Wms 410*	5	4	80.00	0.49	5	2.45	250-370
10	*Wms 155*	3	3	100.00	0.46	3	1.38	20-160
11	*Wms 408*	7	6	85.71	0.33	7	2.31	60-230
12	*Wms 249*	4	3	75.00	0.47	4	1.88	130-185
13	*Wms 437*	6	5	83.33	0.34	6	2.04	50-135
14	*Xbarc 314*	2	1	50.00	0.29	2	0.58	210-260
15	*Wms 443*	6	6	100.00	0.38	6	2.28	100-240
16	*Wms 95*	5	4	80.00	0.49	5	2.45	40-135
17	*Wms 114*	5	5	100.00	0.49	5	2.45	70-200
18	*Wms 94*	3	3	100.00	0.34	3	1.02	80-160
19	*Wms 44*	5	5	100.00	0.48	5	2.40	50-200
20	*Wms 112*	9	8	88.89	0.46	9	4.14	50-250
Total		103	96	93.20	8.27	103	851.81	
Mean		5.15	4.8	4.66	0.41	5.15	42.59	

bp; base pair, **n_a:** observed number of alleles, **PIC**: polymorphism information content, **MI**: Marker Index; **EMR**: Effective Multiplex Ratio containing 17 wheat genotypes was calculated using 20 SSRs and a model based approach of STRUCTURE. For estimation of the exact population substructure (K), ten independent Ks (from K=2 to K=10 where, K is kinship matrix). The STRUCTURE analysis divided the population into four main groups (Figure 4), but the differentiations at K=3 were almost consistent with pedigree knowledge with few exceptions. Thus, the pedigree information was used to guide the division of P_1, P_2, P_3 groups combining with the cluster membership.

The P_1 group consisted of 6 genotypes, P_2 with three genotypes, P_3 with three genotypes, whereas admixture group designated as P_4 consisted of five genotypes. The genotypes generated by DARwin software were further validated by STRUCTURE analysis at K=3. The kinship analysis indicated most lines had no or weak relationship with the other lines in this wheat panel, which agreed with various sources of the collected lines. However, with the help of a structure bar plot (Figure 4), admixture was recorded and genotypes were separated in three different gene pools represented purity membership of 55.55 percent, 37.5 percent and 37.5 percent for P_1, P_2 and P_3 groups, respectively. However, P_4 showed the presence of genotypes, depicting the estimated membership of each genotype in each of the populations, able to know about the introgression in the genotypes in the present population of wheat there by explaining the grouping better than a dendrogram. Genotypes sharing the same gene pool may be due to the similarity in morphological traits such as genotypes falling in P_1 gene pool have taken 181 to 190 days to 75 per cent maturity, genotypes sharing P_2 gene pool were absent in anthocyanin coloration of auricle, green plant foliage colour, long plant height and medium flag leaf length. Similarly, genotypes sharing the P_3 gene pool were long in flag leaf length and genotypes in P_4 representing the admixture groups were similar in traits such as long plant height, semi-erect plant flag leaf attitude and medium flag leaf width. Earlier [7] performed work on 319 Indian wheat varieties to assess the status of genetic diversity among them. Jaccob's similarity model and Bayesian analysis were performed using software package DARwin 5.0 and STRUCTURE 2.3.3, respectively. Genetic variability parameters computed using POPGENE 1.32.

Cluster and PCA Analyses

Based on polymorphism exhibited by SSR markers, dendrogram was constructed using Jaccard's similarity coefficient using UPGMA method of NTSYS-PC package (version 2.02), the genotypes were grouped into three major clusters (Figure 1) with a genetic similarity of 37-67 per cent. Cluster A was sub-grouped into two clusters A_1 and A_2 comprised of six and three genotypes, respectively, six genotypes were placed in cluster B (2 in subcluster B_1 and four in subcluster B_2). Cluster C comprised of two genotypes HPW 236 and HS 295.

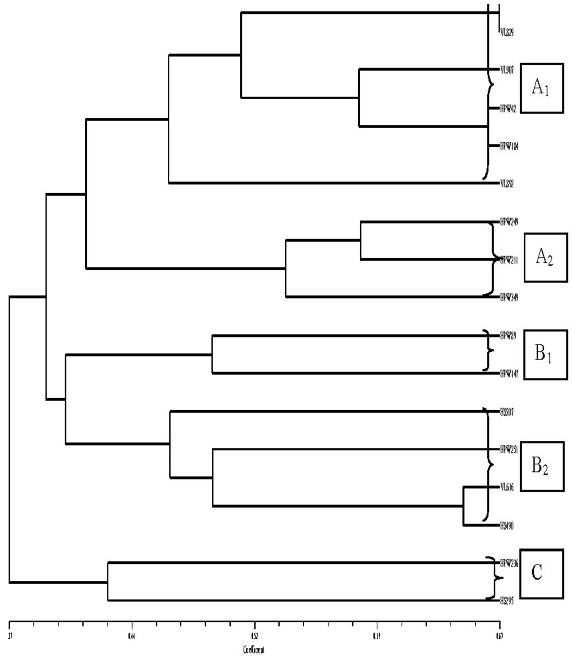

Figure 1. Dendrogram based on morphological traits constructed using squared Euclidean distance and group average clustering method

Clustering of population into three distinct groups represents the between population diversity and indicates a significant influence of environment on genetic diversity. In sub-clusters, different populations with different regional origin were classified into same cluster. It seems there was slight genetic difference between these populations grouped in the same sub-cluster. It mainly happens for population evolved in environments which differs slightly from each other in terms of climatic conditions. This was in confirmation with the results [47]. Using SSR data, neighbour-joining tree was computed using the DARwin software version 5.0.158 [48]. Branch robustness was tested using 1000 bootstraps. Here, also SSR analysis resulted in almost similar grouping of the test population with the Jaccard's similarity coefficient using UPGMA method.

Effectiveness of SSR markers in the genetic diversity analysis of wheat genotypes has been well documented

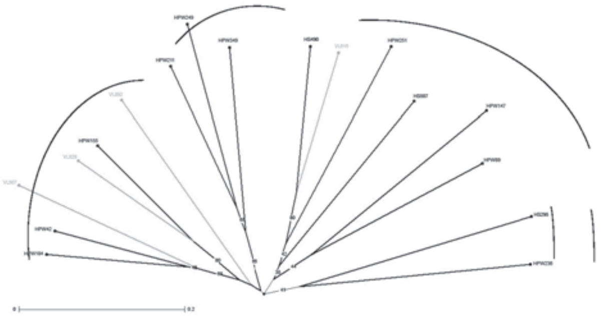

Figure 2. Radial neighbor-joining tree based on 103 alleles from 20 SSR loci among 17 wheat cultivars

[7]. Analysis of [49] 45 varieties of wheat at between 7-9 different SSR loci, and 10 varieties of tomato were analyzed at six loci. The results showed that there was variation between both varieties and microsatellites in the degree of non-uniformity observed, and it was possible to identify a number of different probable sources of non-uniformity. Work [50] on distinctness and uniformity evaluation of wheat (*Triticum aestivum* L.) varieties in Henan was recorded by SSR molecular analysis. The results showed that the discrimination power of SSR molecular markers to wheat varieties had significant positive correlations to polymorphism loci. The cluster tree generated with the described microsatellite markers recognized three major clusters with minimum 37 per cent genetic similarity. Cluster A includes nine genotypes, cluster B includes six genotypes and cluster C include two genotypes. Earlier studies [47] examined genetic diversity of 12 wheat genotypes using four simple sequence repeats. Cluster analysis based on microsatellite allelic diversity and by using UPGMA cluster tree analysis led to the grouping of 12 wheat varieties into three major clusters. Earlier researches [51] selected ten simple sequence repeat (SSR) marker sets an evaluated them in a total of sixteen accessions of bread wheat (*Triticum aestivum* L.). According to cluster analysis, the studied genotypes distributed into two major groups. The groups I and II consist of four and three subgroups, respectively.

Principal component analysis is used to explain genetic variation, show the variation pattern in a multidimensional pattern and do a better interpretation of the relationship between individuals. The relative variance of each component indicates the importance of the related component of total variance and is expressed as a percentage. Clustering patterns in PCA was in correspondence with clustering of both UPGMA tree and the STRUCTURE. Principal component analysis as well as population structure have been shown to be good predictors of grouping patterns and they can be used to complement the clustering method analysis, since different combinations of genetic distance matrices and clustering algorithms can give rise to somewhat different groups [52]. Principal Coordinate Analysis (PCoA) was used to analyse substructures of wheat genotypes belonging to Himachal Pradesh, (ICAR) Shimla and (VPKAS) Almora (Figure 3). Using SSR markers, first principal component explained 37.25 per cent of the total variation, second component explained 8.93 per cent variation, third component explained 8.83 per cent variation, fourth component explained 6.85 per cent variation and the fifth one explained 6.01 per cent variation of the data. Most individuals within a region were grouped more closely. Genotypes of HPW series and HS series were located quite distant from each other.

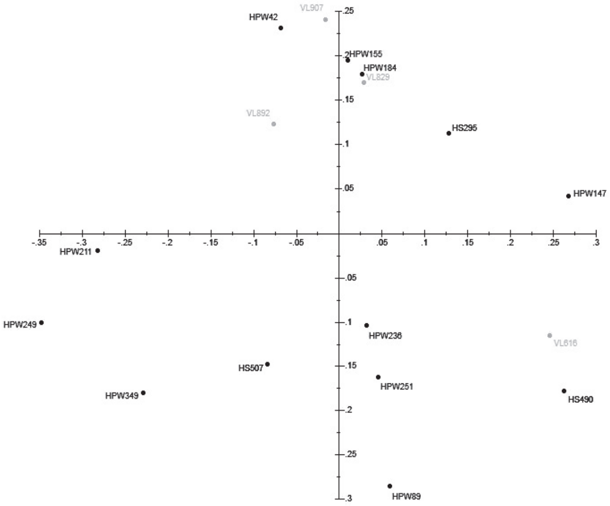

Figure 3. Principal Coordinate Analysis (PCoA) using SSR markers

The genotypes from the HPW series and HS series were more distant, indicating that higher genetic diversity resided in them. Whereas, some of the genotypes of HPW series *viz.,* HPW 349, HPW 249 and HPW 211 fall in same cluster group generated by NTSYS–PC (version 2.02) using UPGMA method, in clusters formed by neighbour-joining tree using DARwin software and also in same quadrangle in principal component analysis depicting that these genotypes were similar to each other but diverse from the rest of genotypes of same series. Clustering of population into distinct groups represents the diversity between populations and indicates a significant influence of environment on genetic diversity.

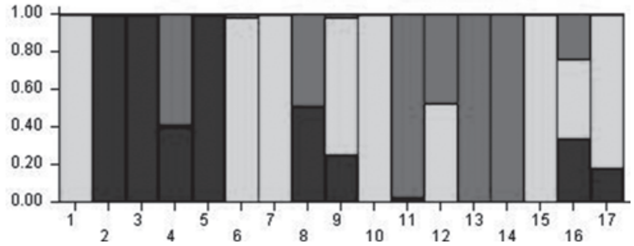

Figure 4. Genetic structure of 17 wheat cultivars as inferred by STRUCTURE v2.3.3 with 20 SSR markers data set

Legend: 1: HPW155; 2: HPW249; 3: HPW349; 4: HPW89; 5: HPW211; 6: VL829; 7:VL907; 8: HS507; 9:VL892; 10:HPW42; 11: HPW251; 12:HPW147; 13:VL616; 14: HS490; 15: HPW184; 16: HPW236; 17: HS295

In sub-clusters, several local populations with different regional origin were classified into same cluster. It seems that there was slight genetic difference between these populations grouped in the same sub-cluster. Such a classification may reflect gene flow among different maize population in different regions or environments. The discrepancy between the known pedigree of some genotypes and the dendrogram placement could be due to relatively low number of SSRs used.

CONCLUSION

The morphological and molecular analysis revealed considerable diversity among the genotypes studied. Furthermore, the congruence of molecular markers with the morphological descriptors provides a powerful tool to characterize and identify the genotypes of wheat. Three genotypes *viz*., HPW 249, HPW 349 and HPW 211 collectively formed a different cluster in each diversity analysis (on the basis of morphological and molecular data) showing that they were diverse from the other genotypes but similar to each other. Cluster III showed the maximum cluster mean values in D^2 analysis for maximum number of traits, suggesting that these traits were superior over other traits and genotypes falling in cluster III would be selected directly on the basis of these traits and could be used in identification programme. HPW 249, HPW 349 and HPW 211 were found to fall under a single cluster in D^2 analysis and forming a separate sub clusters in molecular analysis as well. So this genotype could be used in for future breeding programmes for genetic improvement in wheat.

REFERENCES

1. ESQUINAS-ALCÁZAR J (2005). Protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nature Reviews Genetics*, **6**: 946-953.
2. SMITH OS AND JSC SMITH (1992). Measurement of genetic diversity among hybrids: a comparison of isozymic, RFLP, pedigree, and heterosis data. *Maydica,* **37**: 53-60.
3. AMSELLEM L, C DUTECH AND N BILLOTTE (2001). Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir. (Rosaceae), an invasive weed in La Re´union Island. *Molecular Ecology Notes,* **1**: 33-35.
4. DANQUAH EY, DE JOHNSON, C RICHES, GM ARNOLD AND A KARP (2002). Genetic diversity in *Echinochloa* sp. from different geographic origins and within rice fields in Coˆte d'Ivoire. *Weed Research,* **42**: 394-405.
5. KIM HK, L SEUKKI, MJ SEO, HO K LEEAGI, S CHUN AND JJ MOON (2014). Genetic diversity and population structure of wild soybean (*Glycine soja* Sieb. and Zucc.) accessions in Korea. *Plant Genetic Resources,* **12**: 45-48.
6. NACHIMUTHU VV, R MUTHURAJAN, S DURAIALAGURAJA, R SIVAKAMI, BA PANDIAN, G PONNIAH, K GUNASEKARAN, M SWAMINATHAN, KK SUJI AND R SABARIAPPAN (2015). Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa. Rice,* **8**: 30-37.
7. ARORA A, S KUNDU, N DILBAGHI, I SHARMA AND R TIWARI (2014). Population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers. *Australian Journal of Crop Science,* **8**: 1281-1289.
8. SHARMA, RK, P GUPTA, V SHARMA, A SOOD, T MOHAPATRA AND PS AHUJA (2008). Evaluation of rice and sugarcane SSR markers for phylogenetic and genetic diversity analyses in bamboo. *Genome*, **51**: 91-103.
9. AHMAD, S, M SINGH, ND LAMB-PALMER, M LEFSRUD AND J SINGH (2012). Assessment of genetic diversity in 35 *Pisum sativum* accessions using microsatellite markers. *Canadian Journal of Plant Science,* **92**, 1075-1081.
10. HAMMER K, AA FILATENKO AND V KORZUN (2000). Microsatellite markers - a new tool for distinguishing diploid wheat species. *Genet. Resour. Crop Evol*., **47**: 497-505.
11. BORNER A, S CHEBOTAR AND V KORZUN (2000). Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. *Theoretical Applied Genetics*, **100**: 494-497.
12. HUANG Q, A BORNER, S RODER AND W GANAL (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical Applied Genetics*, **105**: 699-707.
13. ANONYMOUS (2007). Guidelines for the conduct of test for Distinctiveness, Uniformity and Stability. Protection of Plant Varieties and Farmer's Right Authority (PPV & FRA), Government of India.
14. MANSING JS (2010). Characterization of wheat (*Triticum* sp.) genotypes through morphological, chemical and molecular markers. M. Sc. Thesis. Department of Seed Science and Technology, University of Agriculture Sciences, Dharwad, Karnataka, India.
15. PANSE VG AND PV SUKHATME (1984). Statistical Methods for Agricultural workers. ICAR, New Delhi. p: 359.
16. BURTON GW AND EW DE VANE (1953). Estimating heritability in tall Fescue (*Festuca arundinacea*) from replicated colonal material. *Agronomy Journal,* **45**: 478-481.

17. JOHNSON HW, HF ROBINSON AND RE COMSTOCK (1955). Estimates of genetic and environmental variability in soybeans. *Agronomy Journal,* **47**: 314-318.

18. MAHALANOBIS PC (1936). On the generalized distance in statistics. *Proceedings of the National Academy of Sciences*, **2**: 49-55.

19. DOYLE JJ AND JE DOYLE (1990). Isolation of plant DNA from fresh tissue. *Focus*, **12**: 13-15.

20. SHARMA V, P BHARDWAJ, R KUMAR, RK SHARMA, A SOOD AND PS AHUJA (2009). Identication and cross-species amplication of EST derived SSR markers in different bamboo species. *Conservation Genetics*, **10**: 721-724.

21. BOTSTEIN D, KL WHITE, M SKOLNICK AND RW DAVIS (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32**: 314-331.

22. YEH FC AND TJB BOYLE (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*, **129**: 157.

23. ROHLF FJ (1998). NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.0. Applied Biostatistics Inc., Setauket, New York. 1998, 37.

24. PERRIER X AND JP JACQUEMOUD-COLLET (2006). DARwin software.

25. PRITCHARD JK, M STEPHENS AND P DONNELLY (2000). Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945-959.

26. FALUSH D, M STEPHENS AND JK PRITCHARD (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**: 574-578.

27. EVANNO G, S REGNAUT AND J GOUDET (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611-2620.

28. EARL DA AND BM VONHOLDT (2011). Structure Harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources*, **4**: 359-361.

29. RANA JC AND GUPTA VP (1993). Genetic analysis of some physiological traits in pea. *Indian Journal of Pulse Research*, **6**(1): 38-44.

30. ZAHIR A, SQ AFSARI, A WAQAL, G HASEENA, N MOHAMMAD AND G ABDUL (2007). Evaluation of Genetic Diversity Present in Pea (*Pisum Sativum* L.) Germplasm Based on Morphological Traits, Resistance to Powdery Mildew and Molecular Characteristics. *Pakistan Journal of Botany*, **39**(7): 2739-2747.

31. PACHAURI V, N NILAY TANEJA, P VIKRAM, NK SINGH AND S SINGH (2013). Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). *Australian Journal of Crop Science,* **7**: 923-932.

32. ZONG X, RJ REDDEN, Q LIU, S WANG, J GUAN, J LIU, Y XU, X LIU, J GU, L YAN, P ADES AND R FORD (2009). Analysis of a diverse global *Pisum* sp. collection and comparison to a Chinese local collection with microsatellite markers. *Theoretical Applied Genetics*, **118**: 193-204.

33. JING R, MA AMBROSE, MR KNOX, P SMYKAL, M HYBL, A RAMOS, C CAMINERO, J BURSTIN, G DUC, LJM VAN SOEST, WKS WIE²CICKI,, MG PEREIRA, M VISHNYAKOVA, GF DAVENPORT, AJ FLAVELL AND THN ELLIS (2012). Genetic diversity in European *Pisum* germplasm collections. *Theoretical Applied Genetics*, **125**: 367-380.

34. RAWASHDEH NASAB K, H NASRI, MA MOHAMAD AND T MUNIR (2007). Phenotypic diversity of durum wheat (*T. durum* Dest.) from Jordan. *Genetic Resources and Crop Evaluation,* **54**: 129-138.

35. ZAHARIEVA M, A DIMOV, P STANKOVA, J DAVID AND P MONNEVEUX (2003). Morphological diversity and potential interest for wheat improvement of three *Aegilops* L. Species from Bulgaria. *Genetic Resources and Crop Evolution*, **50**: 507-517.

36. KARAGOZ A, N PILANALI AND T POLAT (2006). Agro-morphological characterization of some wild wheat (*Aegilops* L. and *Triticum*) species. *Turkish Journal of Agriculture,* **30**: 387-398.

37. NAGHAVI MR, SR MONAFARED, AH AHKAMI AND MA OMBIDBAKHSH (2009). Genetic variation of durum wheat landraces and cultivars using morphological and protein markers. *World Academy of Science, Engineering and Technology,* **49**: 73-75.

38. LIMA-BRITO J, A CARVALHO, A MARTIN, JS HESLOP-HARRISON AND H GUEDES-PINTO (2006). Morphological, yield, cytological and molecular characterization of a bread wheat×*tritordeum* F_2 hybrid. *Journal of Genetics,* **85**: 123-131.

39. DEGEWIONE A, T DEJENE AND M SHARIF (2013). Genetic variability and traits association in bread wheat (*Triticum aestivum L*.) genotypes. *International Research Journal of Agriculture Sciences,* **1**: 19-29.

40. HOKRANI V, V RUDRANAIK, HL NADAF, SA DESAI, SK DESHPANDE, IK KALAPANAVAR AND BN PATIL (2013). Genetic studies in free threshable advanced segregating population of tetraploid wheat (*Triticum dicoccum*). *Karnataka Journal of Agriculture Science,* **26**: 10-13.

41. KUMAR N, S MARKER AND V KUMAR (2014). Studies on heritability and genetic advance estimates in timely sown bread wheat (*Triticum aestivum L*.). *Bioscience Discovery,* **5**: 64-69.

42. MAJUMDAR DAN, AKM SHAMSUDDIN, MA KABIR AND L HASSAN (2008). Genetic variability correlated response and path analysis of yield and yield contributing traits of spring wheat. *Journal of Bangladesh Agriculture University,* **6**: 227-234.

43. STRUSS D AND J PLIESKE (1998). The use of microsatellite markers for detection of genetic diversity in barley population. *Theoretical Applied Genetics*, **97**: 308-315.

44. SOSINSKI B, ZX LU, A TABB, K SOSSEY-ALAOUI, S RAJAPAKSE, K GLASSMOYER, R SCORZA, G REIGHARD, RE BALLARD, WV BAIRD AND AG ABBOTT (1998). Use of AFLP and RFLP markers to create a combined linkage map in peach (*Prunus persica* (L.) Batsch) for use in marker assisted selection. *Acta Horticulture,* **465**: 61–68.

45. PRASAD M, RK VARSHNEY, JK ROY, HS BALYAN AND PK GUPTA (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theoretical and Applied Genetics,* **100**: 584-592.

46. FALUSH D, M STEPHENS AND JK PRITCHARD (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics,* **164**: 1567-1587.

47. ISLAM S, MS HAQUE, RM EMON, MM ISLAM AND SN BEGUM (2012). Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. *Bangladesh Journal of Agricultural Research*, **37**: 389-398.
48. PERRIER X AND JP JACQUEMOUD-COLLET (2006). DARwin software.
49. COOKE RJ, GMM BREDEMEIJER, MW GANAL, R PEETERS, P ISAAC, S RENDELL, J JACKSON, MS RODER, V KORZUN, K WENDEHAKE, T ARESHCHENKOVA, M DIJCKS, D LABORIE, L BERTRAND AND B VOSMAN (2003). Assessment of the uniformity of wheat and tomato varieties at DNA microsatellite loci. *Euphytica***, 132**: 331-341.
50. HAN FENGLONG LI, ZHENGLING HU LIN AND XU WEIGANG (2010). Distinctness and uniformity evaluation of wheat (*Triticum aestivum*) varieties in Henan by SSR molecular analysis. *Scientia Agricultura Sinica,* **43**: 3698-3704.
51. HAMDALLA MS (2014). Assess the degree of genetic diversity among sixteen complexes genetically wheat bread using SSR indicators. *International Journal of Scientific and Technology Research,* **3**: 19-23.
52. REIF JC, ML WARBURTON AND XC XIA (2006). Grouping of accessions of Mexican races of maize revisited with SSR markers. *Theoretical Applied Genetics,* **113**: 177-185.