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Genetic diversity analysis based on morphological traits and microsatellite markers in barley (*Hordeum vulgare*)

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ABSTRACT

The experimental material consisting of 10 barley (*Hordeum vulgare* L.) cultivars were evaluated using biometrical technique and microsatellite markers. A set 19 morphological and 47 SSR markers revealed significant differences among the quantitative and qualitative traits. In total 166 alleles were scored from PCR based amplification profiles for selected genotypes. The number of alleles ranged from 2 to 7 with an average of 3.52 alleles per locus. The band fragment size varied from 100 bp to 400 bp with PIC values ranging from 0.286 (Bmac154) to 0.804 (KV1/KV2) with an average of 0.551. Genetic diversity was estimated using Euclidean^2 distance and UPGMA methods. Euclidean clustering revealed four clusters and groups II, III and IV exhibited highest mean value for most of the traits. Genotypes JB 1, LAKHAN, KB 1201 and RD 2035 were identified as most suitable genotypes for hybrid performance for yield attributing characters like tillers/plant, grains/spike, 1 000 grain weight, grain weight/spike, grain size and days to maturity. This information would be use as selection parameters to plan crosses and maximize the use of genetic diversity and expression of heterosis in barley breeding program for yield improvement.

Key words: Biometric traits, Cluster analysis, Genetic diversity, SSR markers

Presence of genetic diversity and relationships within genotypes is a prerequisite and important step in the development of new cultivars. Development of hybrid varieties with desirable traits requires a thorough knowledge about the existing genetic diversity in available germplasm (Singh *et al*. 2014). Higher heterotic expression in F_1s and broad spectrum of variability in segregating population requires to identify the more genetically diverse parents (Shekhawat *et al*. 2001). If superior crosses could be predicted before field evaluation, the efficiency of hybrid breeding programs could be greatly enhanced (Benin *et al*. 2012). Different criteria like morphological, biochemical and molecular markers can be utilized for the genetic diversity studies (Cox *et al*. 1985).

Some studies on genetic diversity of barley (*Hordeum vulgare* L.) have focused on phenological and quantitative traits using different methods like Mahalanobis's generalized distance (D^2) (Shakhatreh *et al*. 2010), Principal component analysis (Záková and Benková 2006) and Euclidean ^{2} Distance (Muhe and Assefa 2011). Evans *et al.*, 2010 described variability in barley using analytical determinations of the malting quality. The comparative study based on these different methods for estimating genetic diversity can describe their usefulness in plant breeding program (Almanza-Pinzón *et al*. 2003). The combination of molecular techniques and biometrical methods has allowed the development of more precise way for evaluating genetic diversity and proved to be a powerful tool for unravelling intraspecies taxonomy of barley (Benin *et al*. 2012, Eshghi *et al*. 2012, Abdellaoui *et al*. 2010, Wang *et al*. 2010).

Molecular markers are powerful tools in the characterization and evaluation of genetic diversity within and between genetic populations (Russel *et al*. 1997). They have the advantage of providing thorough genome assessments that are not influenced by the environmental factors. With the development of different molecular techniques such as simple sequence repeats (SSR), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP), single nucleotide polymorphism (SNP) etc., these molecular markers are being regularly used for genotype identification, genetic mapping and marker assisted selection in barley (Feng *et al*. 2006, Kojima et al. 2007). Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have proven to be the markers of choice for genetic diversity studies (Hua *et al*. 2015, Wang *et al*. 2010, Feng *et al*. 2006). Though biometrical traits are often influenced by G×E interaction (Spanic *et al*. 2012) still combined use of morphological and molecular markers allows more representative sampling of the genome

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(Mohammadi and Prasanna 2003). This strategy resulted in extensive studies on combined traditional and molecular marker approach for studying genetic variability in crops like wheat (Salem *et al* 2008), faba bean (Ammar *et al*. 2015) and in maize (Beyene *et al*. 2005) including barley (Amabile *et al*. 2013 and Koebner *et al*. 2003). This study was therefore, conducted to evaluate genetic distances among barley genotypes using their phenotypic and molecular (DNA) variations for their associations with hybrid performance for various quantitative and qualitative traits.

MATERIALS AND METHODS

Total 10 barley cultivars were chosen for this study on the basis of their yield potential and agronomical traits. Cultivars name, their parentage and releasing centers are given in (Table 1). The experiment was carried out in randomized block design with three replication during *rabi* season for two consecutive seasons (2012-14) at Instructional farm, College of Agriculture, Rewa (MP). The experimentation area comes under semi-arid having subtropical climate located at 24.31^0N latitude and 81.15^0 E of longitudes, salty clay loam soil with uniform topography. The experimental plots consisted of 6 rows of 3.0 m length with 23 cm spaces and they were sown by hand. The plant density of 120 plants/m^2 and recommended dose of fertilizer (60:30:20, NPK) kg/ha were applied. Weeds were removed by hand prior to flowering stage. Standard cultural practices were followed for raising the crop.

Data was recorded on 10 randomly competitive plants from the middle row for each cultivars for phenotypic traits, viz. days to 50% flowering, days to maturity, plant height (cm), flag leaf area (cm^2), upper leaf area (cm^2), tillers/plant, spikes/plant, spike length(cm), grains/spike, spike weight (g), grain weight/spike (g), spike harvest index,1 000 grain weight (g), biological yield/plant (g), grain yield/plant (g), harvest index, grain size (mm^2), husk content (%) and protein content (%).

Data scored over morphological traits were subjected to statistical analyses. Genetic parameter of variability, estimation of heritability and genetic advance were computed as per methods suggested by Johnson *et al*. (1955) for understanding the transmissibility of characters. The D^2 value was calculated by method described by Mahalanobis (1936) and standardized Euclidean² distance were performed using the Windostat Software Package Version 9.2 (2015).

Table 1 Details of 10 genotypes used in the study

Variety	Parentage	Breeding/source location
K508	K394/K141	CSAU & T, Kanpur, UP
JB203	JB58XRD2552	JNKVV, Rewa, MP
RD2811	RD2579/JB26/RD2552	SKRAU, Durgapur, Rajasthan
RD2869	RD2670/RD2508/RD2715	SKRAU, Durgapur, Rajasthan
HUB113	KARAN280/C138	BHU, Varanasi, UP
JB1	LAKHANXPL552	JNKVV, Rewa, MP
RD2035	RD103/PL101	SKRAU, Durgapur, Rajasthan
KB1201	K560/K475	CSAU & T, Kanpur, UP
LAKHAN	K12/IB226	CSAU & T, Kanpur, UP
JYOTI	K12/C251	CSAU & T, Kanpur, UP

Equal number of fresh, young leaves (two weeks old seedlings) of at least six plants from each genotype was bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai Maroof *et al*. 1984). The DNA samples were analyzed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis.

Total 47 SSR markers were selected from a set of 125 markers on the basis of their amplification quality and discrimination efficiency during initial screening (Table 2). These markers were randomly located on all the seven chromosomes of barley. PCR reaction was conducted in a reaction volume of 20µl containing 1X PCR buffer. 200 m M dNTPs, 0.25µM of primer, 2Mm $MgCl_2$, 1u Taq polymerase and 50 ng template DNA. PCR amplification was performed using BIORAD S 1000 thermocycler. PCR products were resolved by electrophoresis on 2% agarose gels (HiMedia) at 4v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bpDNA ladder (NEB, UK) and corroborated with the reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by reamplification under similar PCR conditions. Gels were stained with ethidium bromide (0.5ug/ml) and DNA banding patterns were visualized under UV light (Syngene Synoptics Ltd, USA).

Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus, major allele frequency and polymorphism information content (PIC) values were determined (Anderson *et al*. 1993) as shown in (Table 2). Allele molecular weight data were also used to export the data in binary format (allele presence = "1" and allele absence = "0") for analysis with NTSYS-PC version 2.1. The 0/1 matrix was used to calculate genetic similarity as DICE coefficient using SIMQUAL subprogram and the resultant similarity matrix was employed to construct dendrogram using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in NTSYS-PC (version 2.1) to infer genetic relationships and phylogeny. For estimating the similarity matrix, null alleles were treated as missing data to reduce the biased genetic or similarity measures.

RESULTS AND DISCUSSION

Morphological characterization

The analysis of variances revealed significant differences among the genotypes for all the characters studied indicating presence of sufficient genetic variability among the genotypes. The estimates of phenotypic

Table 2 Allelic variation of the polymorphic SSR loci in barley genotypes on the basis of allele richness and PIC

Primers's name	Chr	Sequence (5'-3')	Tm (c°)	No. of alleles	Allele size range (bp)	High frequency allele	% of high frequency allele	PIC
ABG55	1H	ATCCGCAGCAGATCGAGGAC	60	3	300-400	340	50	0.545
Scssr10477	1H	AGAGCAATGAGCTCCTACCC	55	2	170-260	260	60	0.305
GBM1407	1H	AAACCATGCATTCTTCAGAGA	55	3	170-190	180	50	0.572
Bmag579	1H	CCTAGATAAGGAACATAGCCA	55	4	100-160	110	50	0.569
Bmac154	1H	CTGGGTGATGAATAGAGTTTC	58	2	100-130	100	70	0.286
KV1/2HOR1	1H	CCACCATGAAGACCTTCCTC	58	7	250-400	350	30	0.804
Bmac 501	1H	ACTTAAGTGCCATGCAAAG	58	5	100-200	140	70	0.534
Bmac213	1H	ATGGATGCAAGACCAAAC	58	4	150-200	160	70	0.582
Bmag105	1H	AATCAGACCCATCAGAGGT	55	4	100-150	120	40	0.667
Bmag692	2H	GCAAGGTATCTCTTGTATTTTG	55	3	100-200	140	50	0.492
Bmac129	2H	ACTGCATGATAGTATATGGAACA	58	3	110-200	160	50	0.548
EBmatc39	2H	TAGTCTCTTCATTTATACCATCACC	55	2	110-180	160	30	0.375
Bmag 877	3H	AAAGCTCATGGTAGATCAAGA	55	3	100-200	130	50	0.548
Ebmac 541	3H	ACGGATCTACTTTAGCTAGCA	58	4	100-180	110	60	0.535
Bmag225	3H	AACACACCAAAAATATTACATCA	58	4	100- 190	110	40	0.605
Bmac67	3H	AACGTACGAGCTCTTTTTCTA	77	4	100-180	150	40	0.64
ABG500	4H	GCTAGAACTTGACCAATCTC	60	2	110-220	180	40	0.346
Ebmac635	4H	TGCTGCGATGATGAGAACT	58	4	120-200	120	20	0.703
HVM67	4H	GTCGGGCTCCATTGCTCT	TD	2	150-200	200	70	0.332
Ebmac701	4H	ATGATGAGAACTCTTCACCC	55	5	110-200	130	40	0.701
Bmag781	4H	CTATTTTCTAATGCTTGGACC	55	4	130-220	150	40	0.672
Bmag 751	5H	CACTGCAAATATTAAAATGGA	55	3	150-200	180	30	0.535
ABC302	5H	CTGGTGCTTTCGATGCTGT	58	2	300-400	300	50	0.359
Bmag760	5H	GTGATACATCAAGATCGTGC	55	3	140-200	180	50	0.527
Bmag812	5H	ATAGTTCTTTCAGGACCAATG	55	3	110-200	130	60	0.499
Bmag337	5H	ACAAAGAGGGAGTAGTACGC	55	4	130-220	140	40	0.607
Bmag223	5H	TTAGTCACCCTCAACGGT	58	6	130-240	160	30	0.772
Bmag387	5H	CGATGACCATTGTATTGAAG	58	3	100-160	110	50	0.548
Bmag222	5H	ATGCTACTCTGGAGTGGAGTA	58	6	130-210	150	30	0.761
Bmac163	5H	TTTCCAACAGAGGGTATTTACG	55	3	140-200	160	40	0.586
Bmac518	5H	ATATGGGTCACACTGAAAATC	58	6	140-230	160	30	0.761
Bmag 500	6H	GGGAACTTGCTAATGAAGAG	58	4	110-120	110	40	0.687
Bmac40	6H	AGCCCGATCAGATTTACG	58	5	110-230	110	40	0.702
Bmag173	6H	CATTTTTGTTGGTGACGG	58	4	150-230	170	30	0.663
HVM11	6H	CCGGTCGGTGCAGAAGAG	55	3	170-220	170	50	0.527
Bmac18	6H	GTCCTTTACGCATGAACCGT	55	3	150-200	160	40	0.563
Bmag110	7H	ACGAGGAGGGACTAGTACAC	58	3	160-210	160	80	0.385
MGB402	7H		55	3	200-300	290	40	0.568
Bmac167	7H	CATTTCCACTTCAAAATATCC	55	3	200-250	230	50	0.489
Bmag369	7H	CACTAGGCACCAATGACTG	58	2	100-160	140	40	0.346
Bmac64	7H	CTGCAGGTTTCAGGAAGG	58	2	140-190	170	70	0.368
Bmac 162	7H	CATGTGTTGAAATCAGTTTTG	58	5	250-400	280	90	0.697
Ebmac715	7H	GCGAACATTGTCATGTTAGTA	55	2	150-190	190	90	0.371
Bmag 341	7H	TCATGGAGACCGTTGTAGT	55	4	200-280	210	50	0.61
Bmac297a	7H	ATAGAGGGGGTGAAGAATAAC	55	4	100-230	110	60	0.66
Bmac273	7H	ACAAAGCTCGTGGTACGT	55	4	100-200	120	50	0.61
Bmac224	7H	GCATATATACCACCCTTGGT	55	2	150-200	180	60	0.365

coefficient variation (PCV) were higher than genotypic coefficient variation (GCV) for all the traits studied in (Table 3). However, relatively low magnitude of difference was observed between GCV and PCV indicating less environmental influence. Similar kind of result was also reported by Jalata *et al*. (2011).The high PCV and GCV recorded for tiller/plant (28.44, 23.71). Heritability in broad sense ranged from 33% (protein content) to 89% (biological

yield/plant) and the expected genetic advance over mean was ranged from 0.21% (grain weight/spike) to 22.02% (biological yield/plant). Morphological traits having high heritability along with high genetic advance could be emphasized for direct selection. Present findings was also in confirmation with Jalata *et al*. (2011).

In this study, morphological data analysis was to investigate levels of genetic diversity among adapted, elite germplasm. Euclidean^2 similarity matrix determined the extent of genetic relationships among the genotypes studied. The pairwise genetic similarity indices presented in (Table 4) indicated that the cultivar JB1 distantly related to that of KB 1201 with the highest disimilarity index (62.06) while, the cultivar LAKHAN was more closely related to JYOTI with the low disimilarity index (11.11). The range 11.11 to 62.06% showed that the genetic variation is considerably high. If these genotypes carry desirable traits then they can be used for barley improvement programme (Arif *et al*. 2005). Therefore, the Euclidean^2 method provides an adequate power of resolution to discriminate the barley genotypes (Singh *et al*. 2014).

Cluster analysis based on the morphological data assigned the genotype into four groups can be distinguished by truncating the dendrogram at value 20 (Fig 1). The first cluster (denoted I) and include three genotypes K 508, HUB 113 and RD 2869. Another cluster (Cluster II) containing four genotypes JB 203, RD 2811, RD 2035 and KB 1201.Cluster III, includes two genotypes

Table 3 Estimates of genetic parameters for different quantitative and qualitative characters of barley

Character	MSS			Mean	Range		GCV	PCV	h^2 (bs) %	GA as % of mean
	Replication [3]	Treatment [9]	Error [27]		Min.	Max.				
Days to flowering	8.61	96.10***	6.33	74.58	68.00	83.00	7.33	8.07	83	10.24
Days to maturity	1.03	21.24*	6.52	120.60	116.33	126.33	1.84	2.80	43	2.99
Plant height (cm)	36.72	334.24***	15.30	93.57	76.33	111.50	11.02	11.79	87	19.86
Flag leaf area (cm²)	2.71	9.87*	3.46	18.10	15.97	21.50	8.08	13.07	38	1.86
Upper leaf area (cm²)	17.69	36.31**	7.40	25.56	21.20	32.10	12.15	16.15	57	4.81
Tillers/plant	4.59	15.17***	1.94	8.86	6.00	13.00	23.71	28.44	69	3.61
Spikes/plant	10.03	16.68*	5.96	15.47	12.00	18.67	12.22	19.96	37	2.38
Spike length(cm)	0.22	1.47***	0.20	8.37	7.20	9.40	7.77	9.45	68	1.10
Grains/spike	19.45	36.51*	14.53	39.40	34.90	45.67	6.87	11.87	34	3.23
Spike weight (g)	0.02	0.60*	0.14	4.20	3.07	4.63	9.28	12.97	51	0.57
Grain weight/ Spike (g)	0.01	0.09**	0.02	1.74	1.37	2.00	8.37	12.08	48	0.21
Spike harvest index	11.86	13.68*	5.15	41.75	39.67	45.83	4.04	6.77	36	2.07
1 000- grain weight (g)	2.26	13.12***	2.00	40.56	38.23	44.33	4.75	5.89	65	3.20
Biological yield/ plant (g)	14.22	403.38***	16.60	78.90	65.60	97.50	14.39	15.29	89	22.02
Grain yield/ plant (g)	2.46	17.07***	2.83	29.88	26.67	33.20	7.29	9.21	63	3.55
Harvest index	12.85	47.25**	10.11	38.30	33.63	43.77	9.19	12.38	55	5.38
Grain size (mm²)	1.90	18.17*	6.15	16.90	13.07	20.20	11.84	18.86	39	2.59
Husk content (%)	0.28	3.74*	0.86	11.94	9.97	13.40	8.21	11.29	53	1.47
Protein content (%)	1.84	3.05*	1.23	10.03	7.90	11.10	7.76	13.53	33	0.92

h^2 (bs) =Heritability broad sense, GCV=Genotypic coefficient of variation, PCV=Phenotypic coefficient of variation, GA=Genetic Advance. *, **, *** Significant at 5%, 1% and 0.1% probability level respectively, value in parentheses represents degree of freedom.

Table 4 Pairwise genetic similarity coefficient of 10 barley genotypes/cultivar based on Euclidean^2 distance

Genotype	K 508	JB 203	RD 2811	RD 2869	HUB 113	JB 1	RD 2035	KB 1201	Lakhan	Jyoti
K 508	1									
JB 203	33.94	1								
RD 2811	38.33	21.88	1							
RD 2869	18.75	27.76	24.57	1						
HUB 113	14.93	34.01	32.32	24.78	1					
JB 1	59.46	41.53	58.84	58.91	61.12	1				
RD 2035	37.66	26.12	32.11	28.46	27.57	59.04	1			
KB 1201	40.2	28.17	43.22	33.7	44.15	62.06	40.74	1		
Lakhan	44.83	37.37	26.81	40.47	39.92	35.1	43.79	51.46	1	
Jyoti	41.62	38.85	29.19	44.62	39.51	40.35	41.74	48.92	11.11	1

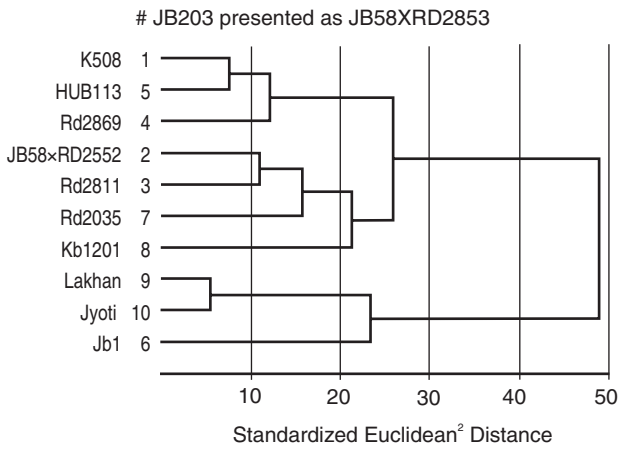

Fig 1 Clustering pattern barley varieties by Euclidean method.

Lakhan and Jyoti. Cluster IV contain only 1 genotype JB 1. The discrimination of genotypes in to discrete clusters supported presence of diversity in the genetic material and the dendrograms showed a good fit between the graphical representation of distances and their similarities matrices. The knowledge about the genetic relationships of genotypes provides useful information to address breeding programmes and germplasm resource management (Rolda´n-Ruiz *et al*. 2001)

Cluster mean values for all characters presented in (Table 5). Cluster II, III and IV exhibited highest mean value for most of the characters. Maximum variance was observed for the major yield component like upper leaf area and grain size (in cluster II) and Flag leaf area, spikes/plant, spike length, spike weight and grain weight/spike (in cluster III) whereas, tillers/plant, grains/spike, 1 000 grain weight and grain yield/plant(in cluster IV). Genotypes with traits of high mean value could be used as parents in hybridization to obtain desirable recombinants. Similar kind of studied have been carried out by (Singh *et al*. 2014, Sharma *et al*. 2008)

Molecular characterization

SSR analysis: Forty seven SSR primer pairs detected 166 alleles in 10 barley samples, with each locus comprising an average of 3.52 alleles. The difference in the number of alleles of different markers was significant. The number of alleles varied from 7 at the KV1/KV2 locus to 2 each for the Scssr10477, Bmac154, EBmatc39, ABG500, HVM67, ABC302, Bmag369, Bmac64, Ebmac715 and Bmac224 loci. And for most the markers it's varied from 3 to 6 (Table 2). These results were comparable with results reported by (Hua W *et al*. 2015). This suggests that significant genomic variability detected by SSR markers among the genotypes. However, there were a number of markers which produced only few alleles and despite their ability to produce only few alleles, they were robust enough to distinguish different accessions of the same genotype. (Russel *et al*. 1997, Abdellaoui *et al*. 2010). The polymorphism information content (PIC) value is a measure of polymorphism among

Table 5 Clusters mean of different quantitative traits among barley genotypes

Cluster	Days 50% to flowering	Days to maturity	Plant height	Flag leaf area (cm^2)	Upper leaf area (cm^2)	Tillers/ plant	Spikes/ plant	Spike length	Grains/ spike	Spike weight (g)	Grain weight/ spike (g)	Spike harvest index	1000 grain weight (g)	Biological yield/ plant (g)	Grain yield/ plant (g)	Harvest index	Grain size (mm^2)	Husk content (%)	Protein content (%)
Cluster I	73.11	121.22	90.26	16.69	24.27	6.90	15.33	8.02	37.69	4.23	1.68	40.04	38.83	72.97	27.59	37.86	13.70	10.57	10.02
Cluster II	72.38	119.33	89.21	18.67	28.30	9.23	15.00	8.00	38.52	4.13	1.72	42.03	39.92	71.16	29.58	41.63	19.02	12.58	10.08
Cluster III	79.00	122.00	108.65	20.10	24.18	9.00	18.17	9.20	40.58	4.35	1.82	41.70	42.57	94.00	32.40	34.52	17.15	12.65	11.00
Cluster IV	79.00	121.00	90.80	16.07	21.20	13.00	12.33	9.20	45.67	4.03	1.80	45.83	44.33	97.50	32.97	33.83	17.53	12.10	7.90
Mean	74.58	120.60	93.57	18.10	25.56	8.86	15.47	8.37	39.40	4.20	1.74	41.75	40.56	78.90	29.88	38.30	16.90	11.94	10.03

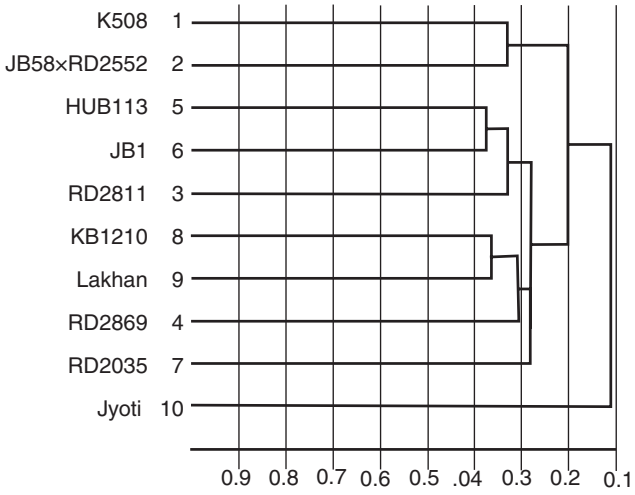

Fig 2 Clustering pattern barley varieties by UPGMA method.

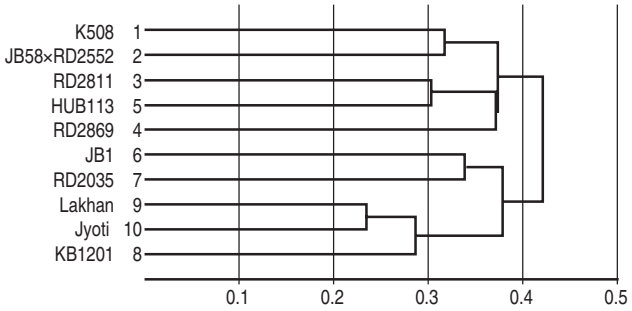

Fig 3 Clustering pattern barley varieties by morphological data analysis coupled with molecular analyses (SSR markers).

genotypes for a marker locus. PIC value greater than 0.50 indicates that the SSR markers enabled a high level of polymorphism (Spanic *et al*. 2012).In this study the PIC value of each marker evaluated on the basis of its alleles, varied greatly from 0.28 (Bmac154) to 0.80(KV1/KV2) with an average of 0.551 (Table 2).

The SSR markers based genetic similarity coefficient for all genotypes was ranged from 0.63 to 0.92, and the two most closely related cultivars were Jyoti and Lakhan with highest similarity coefficient of (0.92). On the other hand, the two most distantly related cultivars were JB1 and HUB113 with low similarity index (0.63), (Table 6). A dendrogram derived from UPGMA cluster analysis based on the genetic similarity (gs) matrix coefficient for the 10 barley genotypes was constructed (Fig 2). Four groups can be distinguished by truncating the dendrogram at gs value of 0.30.The major group (denoted group A) contained four genotypes and includes the barley cultivar KB 1201, Lakhan, RD 2869 and RD 2035. Another group (Group B) consists of three genotypes and includes HUB 113, JB 1 and RD 2811. Group C consists of two genotypes K508 and JB203. Whereas, genotype Jyoti is not clustered to any other genotypes and form a separate group (Group D). It indicated that the genetic dissimilarity between the barley cultivars was comparatively high and Jyoti was the most diverse. Thus, SSR markers provide adequate power to discrimination. In several other studies, diversity analysis in barley using SSR marker have been previously reported (Sonia *et al*. 2004, Guasmi *et al*. 2008, Hajmansoor *et al*. 2010).

Comparative analysis of phenotypic and molecular diversity

Similarity indices and dendrograms were developed on the basis of 19 morphological traits and 47 SSR markers data as shown in (Table 7) and (Fig 3).The genetic similarity coefficient for all genotypes was ranged from 0.53 to 0.77.The similarity indices showed that the two most closely related genotypes were Lakhan and Jyoti with the highest similarity index (0.77). On the other hand, the two most distantly related cultivars JB 203 and KB 1201with low similarity index (0.53) and interestingly, the genotype Jyoti was *on par* to low similarity index with most of the others genotypes except Lakhan and KB 1201, which indicated its genetic dissimilarities.

UPGMA cluster analysis of genetic similarity (gs) matrix resulted in the dendrogram. Three groups can be distinguished by truncating the dendrogram at gs value of 0.45. The major group (denoted group I) consists of 5 genotypes and includes the barley cultivars (K 508, JB 203, RD 2811, HUB 113 and RD 2869) with 0.35 of genetic similarity. Another group (Group III) includes 3 genotypes (Lakhan, Jyoti, KB 1201) with 0.28 of genetic similarity. Group III include 2 genotypes (JB 1 and RD 2035) showed

Table 6 Genetic similarity coefficient of 10 barley genotypes/cultivar based on UPGMA coefficient

Genotype	K 508	JB 203	RD 2811	RD 2869	HUB 113	JB 1	RD 2035	KB 1201	Lakhan	Jyoti
K 508	1									
JB 203	0.67	1								
RD 2811	0.75	0.76	1							
RD 2869	0.78	0.75	0.75	1						
HUB 113	0.80	0.80	0.67	0.78	1					
JB 1	0.78	0.79	0.78	0.74	0.63	1				
RD 2035	0.82	0.84	0.80	0.79	0.72	0.66	1			
KB 1201	0.84	0.89	0.81	0.69	0.72	0.69	0.72	1		
Lakhan	0.82	0.89	0.88	0.80	0.76	0.79	0.79	0.64	1	
Jyoti	0.89	0.92	0.91	0.85	0.81	0.90	0.87	0.74	0.64	1

Table 7 Genetic similarity coefficient of 10 barley genotypes/cultivar based on GOWER'S coefficient

Genotype	K 508	JB 203	RD 2811	RD 2869	HUB 113	JB 1	RD 2035	KB 1201	Lakhan	Jyoti
K 508	1									
JB 203	0.68	1								
RD 2811	0.63	0.63	1							
RD 2869	0.61	0.63	0.63	1						
HUB 113	0.61	0.6	0.7	0.62	1					
JB 1	0.58	0.59	0.59	0.61	0.69	1				
RD 2035	0.56	0.57	0.58	0.59	0.66	0.66	1			
KB 1201	0.55	0.53	0.58	0.66	0.66	0.65	0.65	1		
Lakhan	0.59	0.55	0.56	0.61	0.65	0.62	0.61	0.71	1	
Jyoti	0.55	0.54	0.55	0.58	0.63	0.55	0.56	0.66	0.77	1

genetic similarity of 0.38. Dendrograms showed a good fit between the graphical representation of distances and their original matrices.

The result obtained from different method of divergence study was slightly differs from each other but the clustering patterns of all three methods were different on the basis of the genotypes and their distribution to the clusters. Although, Euclidean^2 statistics classified the genotypes useful in general, but clustering pattern using UPGMA method more critically identifies clusters at different levels. The experimental results were in agreement with the finding of several authors in different crops (Salem *et al*. 2008 in wheat, Ammar *et al*. 2015 in faba bean and Beyene *et al.* 2005 in maize, including barley by Singh *et al*. 2014, Amabile *et al*. 2013, Koebner *et al*. 2003) showed that when marker information was used in conjunction with morphological data, then it classified genotypes better rather that can be done with classifications based on individual data sets.

In this study, morphological data analysis of the barley genotypes was coupled with molecular analyses (SSR markers) to investigate the genetic relationships among 10 barley genotypes. Different method of genetic study adopted to assess the actual genetic potential of genotypes in order to maximize the trait in a predictable manner. Thus, it can be concluded that estimates of genetic similarity based on morphological and molecular markers may provide more accurate genome assessment. Here, Euclidian distance and UPGMA reflected highest diversity between JB 1 and KB 1201, Jyoti and (JB 203), RD 2811 and Jyoti, KB 1201 and (JB 203).

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