

Original Research Article

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## Comparative Analysis of Agro-Morphological and Molecular Variations in Huskless Barley (*Hordeum vulgare* L.) under Central Agro-Climatic Zone of India

P. Banjarey<sup>1</sup>, P. Kumar<sup>1\*</sup>, S. Verma<sup>2</sup>, A.N. Tikle<sup>1</sup>, R. Malik<sup>2</sup>,  
A. Sarker<sup>3</sup> and R.P.S. Verma<sup>3</sup>

<sup>1</sup>R.V.S.K.V.V. (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya) Gwalior-474002, India

<sup>2</sup>ICAR-IIWBR, Karnal-132001, India

<sup>3</sup>ICARDA-IRP (India Research Platform), Amlaha, India

\*Corresponding author

### ABSTRACT

#### Keywords

Huskless barley,  
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#### Article Info

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A set of 24 barley accessions from ICARDA, Lebanon grown under Indian field condition at experimental field of ICARDA- IRP, Amlaha, was analysed for Agro-morphological and molecular variability High (40.11) to moderate (23.58) diversity was observed for all character except plant height (14.60) and ear length (12.84) The PIC values of molecular work in this study ranged from 0.153 to 0.81 with an average of 0.5 indicating sufficient variability among accessions at molecular level. Comparison of clusters of molecular and morphological analysis showed that the accessions from similar or related ancestral background have variability at phenotypic level. Comparative study of diversity at both phenotypic and molecular level provides better information for selection of parents or diverse line for utilization in barley improvement programs.

### Introduction

Barley has been used for various purposes, such as animal feed, malting and brewing, while huskless barley has been used as human food. Barley is one of the cereals with the most diversified genetic basis (Baik and Ullrich, 2008). Based on this knowledge, quantitative and qualitative gains might be obtained in a more effective way during genotype selection and evaluation through appropriate traits, for use in future parent hybridization (Valls, 2007). Knowing the genetic diversity of barley germplasm is necessary for identifying diverse parental

combinations and also creating segregating progeny with high genetic variability for selection. Morphological traits and molecular markers that reveal polymorphism at the DNA level have been shown as a very powerful tool for genotype characterization and estimation of genetic diversity (Salem *et al.*, 2008). Therefore, in the present investigation, accessions of hull-less barley received from ICARDA, were evaluated for agro-morphological and genetic diversity present among them and to classify them in Indian conditions at ICARDA-India Research

Platform (ICARDA-IRP), Amlaha (M.P.). The purpose of this study is to identify best performing accessions to improve production in barley growing areas of central India and for utilization in other breeding programmes for specific purposes. The present study was undertaken to determine the diversity for agro-morphological traits and estimate the genetic distance by molecular markers. This classification of the huskless genotypes will help identification of promising trait-specific genotypes for utilization by breeders in genetic enhancement research, at national and international level.

## **Materials and Methods**

### **Plant material**

The 24 barley accessions from ICARDA, Lebanon grown at experimental field of ICARDA- IRP (India Research Platform), Amlaha, (27°12' N, 77 ° 05' E) in RBD design with 2 replications in 6 row plots of 25 cm distance between rows. Sowing was done on Nov. 12, 2014 during the *rabi* (winter) season of 2014-15. 50% of Nitrogen (30 kg), full dose of Phosphorus (60 kg) and Potassium (40kg) were applied at the sowing as basal dose. Top dressing of remaining Nitrogen was done after 30 days of sowing.

Crop was maintained under rainfed condition. First hand weeding was done with the appearance of thick flush of weeds. Second hand weeding was repeated after two weeks.

### **Phenotypic screening**

Data was recorded for taking 10 randomly competitive plants from the middle row for each accession for nine agro-morphological traits viz. plant height, tillers per plant, days to heading, numbers of spikes per plant, spike length, spikelets per spike, grains per spike, 1000 grain weight and yield per plant.

### **DNA isolation**

Equal number of fresh young leaves (two weeks old seedlings) of at least six from each genotype was bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB plants method (Saghai Maroof *et al.*, 1984).

The DNA samples were analyzed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis.

### **Generation of SSR markers based molecular profiles**

Total 90 SSR markers from different locations of each linkage group of barley genome were selected out and 43 markers showed polymorphism (Table 2).

The necessary details of these primer pairs were obtained from website ([www.wheat.pw.usda.gov/cgi-bin/graingenes.com](http://www.wheat.pw.usda.gov/cgi-bin/graingenes.com)). The PCR reaction was conducted in a reaction volume of 10 µl containing 1X PCR buffer, 200 m MdNTPs, 0.25 µM of primer, 2Mm MgCl<sub>2</sub>, 1u Taq polymerase and 50 ng template DNA. PCR amplification was performed using BIORAD S 1000 thermocycler.

PCR products were resolved by electrophoresis on 2.5% 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 re-amplification 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).

## Data analysis

Cluster analysis was also done through multivariate analysis under the software SPSS (v23). Genetic divergence among the genotypes was assessed by using Euclidean  $D^2$  technique. Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus, and frequency of major alleles were determined. Polymorphic information content (PIC) was calculated for each SSR marker wise estimates of the discriminatory power of locus by taking into account the numbers of alleles that are expressed (Anderson *et al.*, 1993). PIC value were calculated as

$$PIC = 1 - \sum P_{ij}^2$$

Where,  $P_{ij}^2$  is the frequency of the  $i^{th}$  allele.

Allele molecular weight data were also used to export the data in binary format (allele presence = "1" and allele absence = "0") and entered into a matrix. Based on the matrix of (GD) values, SPSS software (v23) was used to obtain the dendrograms, Hierarchical Clustering, depicting Genetic relatedness of the accessions.

## Results and Discussion

Evaluation of the amount of genetic variation in huskless barley germplasm is the essential study for barley breeding. Outcomes of the assessments provide a general guide for choosing parental lines to make suitable cross combinations for particular breeding purposes. In present investigation, 24 genotypes from ICARDA, Lebanon were grown at ICARDA-IRP (IRP), Amlaha (M.P.) to check their morphological diversity in Indian agro-climatic conditions and further evaluate them at molecular level for genetic variation.

## Genetic variability at agro-morphological level

A total of nine agro- morphological characters i.e. plant height, tillers per plant, days to heading, numbers of spikes per plant, spike length, spikelets per spike, grains per spike, 1000grain weight and yield per plant were recorded for analyses. As summarized in table 1, spikelets per spike (75.40), spike length (74.55) and grains per spike (62.20) had shown highest heritability, moderate heritability was observed for tillers per plant (41.20), days to heading (41.12), 1000 grain weight (24.33) and yield per plant (24.33)while, plant height (9.45) and spikes per plant (7.55) had least heritability. For contributing toward divergence, 1000 grain weight (TGW) and yield per plant (Y/P) contributed maximum to divergence with phenotypic coefficient of variance (PCV)40.107 followed by spikelet per spike (S/E) with PCV 36.562. However trait like spike length (EL) and plant height (PH)had contributed least toward divergence with PCV 12.841 and 14.608, respectively. Abebe *et al.*, (2010); Ibrahim *et al.*, (2011); Yadav *et al.*, (2015); have also recorded similar results for these characters for diversity assessment of barley in their studies.

## Clustering based on phenotypic variations

The average of two replications of all the studied characters of the accessions was used to construct a similarity matrix using the SPSS (v23) software package. Though all the accessions developed had same centre of origin, still significant diversity was observed and grouped these genotypes in two major groups for phenotypic traits studied. The dendrogram clustered 18 accessions and 6 accessions into group 2 at G.S. = 0.01 (Fig. 1). The Major group (1) is further divided into two sub groups, A and B, containing 13 and 5 accessions respectively. Present grouping

showed that the accessions having various or distant parents but in same environment didn't show any significant difference in their phenotypic performance. While accessions with either of the same or closely related parent have been clustered in different groups. Ibrahim *et al.*, (2011) also observed grouping of ICARDA genotypes developed from diverse ancestors in same cluster on basis of their morphological traits in given climate.

### **Molecular markers based genetic variability**

Several studies were conducted in world to evaluate the genetic relationships among different barley genotypes using molecular markers (Hou *et al.*, 2005; Ferreira *et al.*, 2016). Pejic *et al.*, (1998) reported that the information of polymorphism would be sufficient if more than 70 alleles were detected. In our study, 43 polymorphic SSR markers randomly distributed across seven chromosomes of barley genome were screened with selected set of genotypes. These markers amplified 119 bands with an average of 2.76 bands per locus (Table 2).

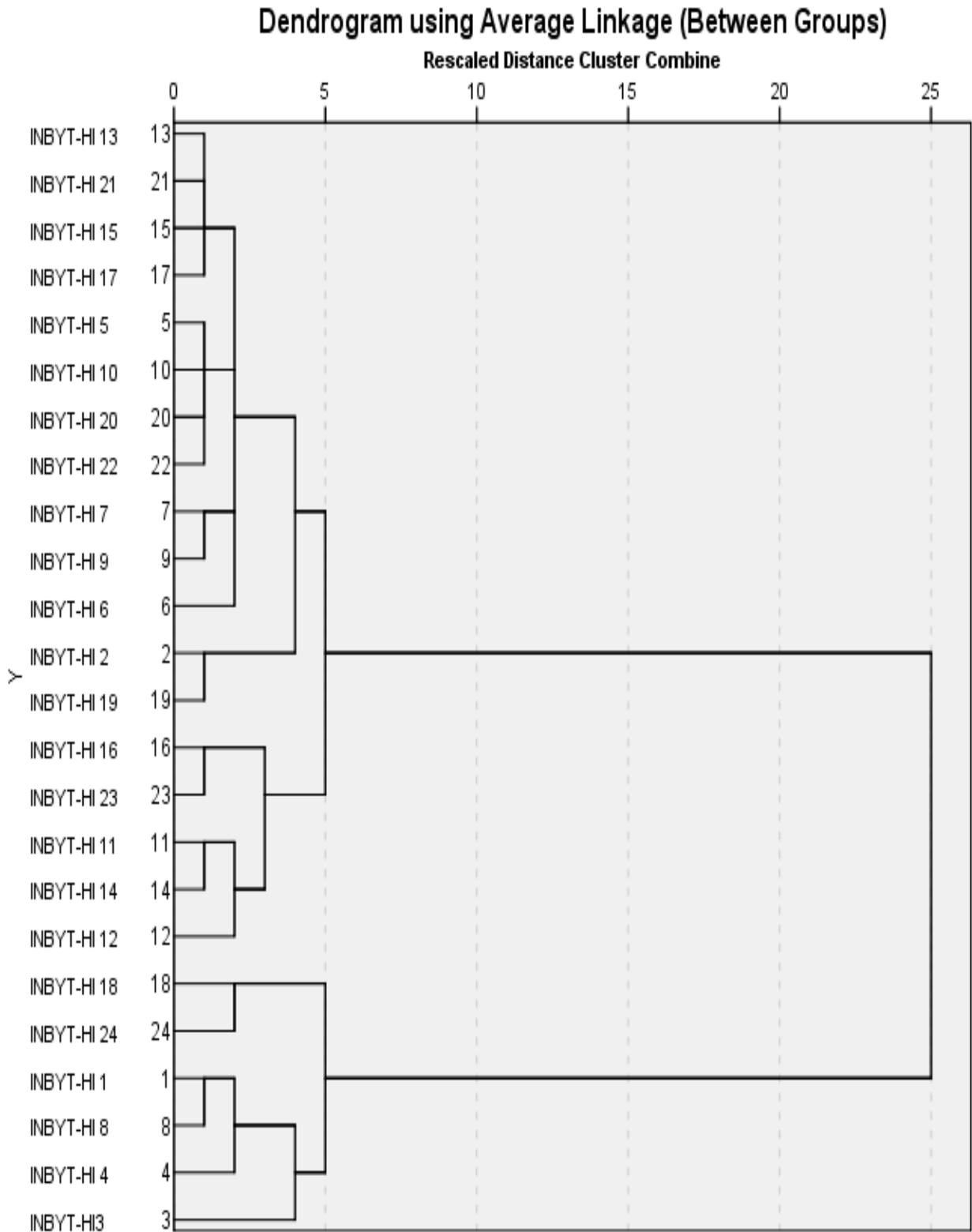
In previous reports, Yadav *et al.*, (2015) and Verma *et al.*, (2015) also reported similar number of alleles in Indian barley during genetic variability studies. Whereas, Ivandic *et al.*, (2002) and Maniruzzaman *et al.*, (2014) reported 5.5 and 7.8 alleles per locus, respectively. Accessions screened in this study are from same centre and their pedigree gene pool is also narrow as there were bred for high yield, therefore, chances of getting fewer alleles per locus are expected and despite this they are robust enough to distinguish different accessions. In our study, chromosome 2 (2H) was found most polymorphic at molecular level across the linkage groups of barley. Previously Yadav *et al.*, (2015) and Verma *et al.*, (2015) also reported similar observations for Indian barley. Spanic *et al.*, (2012) reported that

greater than 0.50 PIC values indicate that SSR markers enable a high level of polymorphism. The PIC values in this study ranged from 0.153 to 0.81 with an average of 0.5 thus indicating sufficient variability in ICARDA accessions at molecular level (Fig. 2). MWG684 marker gave maximum polymorphism with PIC value 0.810 and 5 different allele sizes followed by marker HVM36 and HVM40 with PIC value 0.722, 0.593 respectively and 5 different allele sizes. These results were comparable with results reported by Hua W. *et al.*, (2015), Verma *et al.*, (2015) and Yadav *et al.*, (2015). Yadav *et al.*, (2015) reported PIC range of 0.28 to 0.80 in Indian barley.

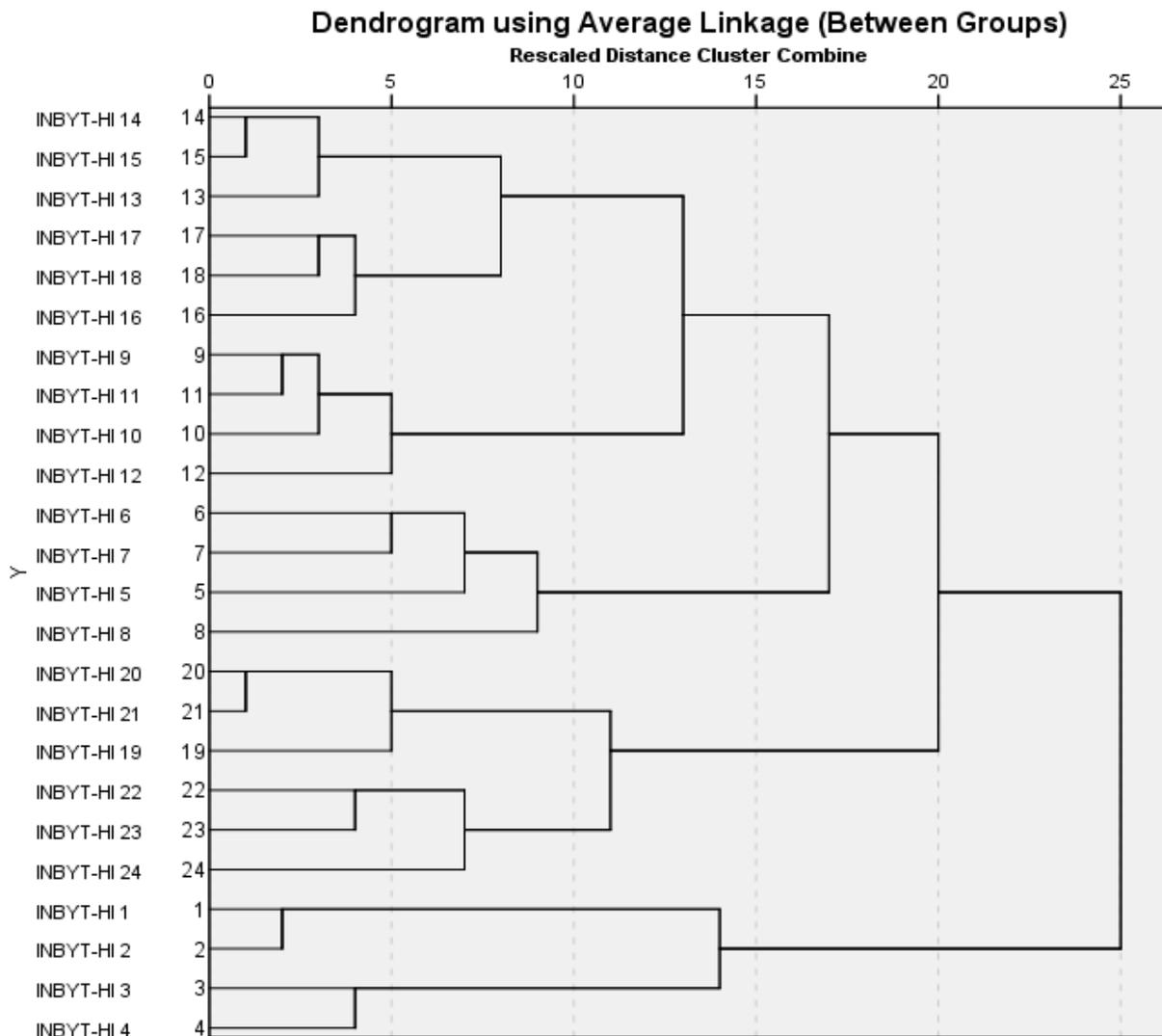
### **Clustering based on molecular screening**

Using binary data of alleles, Sm matrix was generated to develop cluster of accessions based in molecular screening by SPSS software (v23). It revealed that INBYT-HI 14 and INBYT-HI 1 are most similar accessions with highest value of similarity matrix, 0.9075, further accessions INBYT-HI 14 and INBYT-HI 15 with similarity matrix value 0.899 observed to be very closely related. However, maximum distantly related genotypes are INBYT-HI 1 and INBYT-HI 17 with similarity matrix value 0.378 followed by INBYT-HI 1 and INBYT-HI 16 with similarity matrix value 0.386. Genotypes with higher value of similarity matrix had one or another ancestor same in their pedigree. On basis of genetic variability all accessions were clustered into two major groups with 20 accessions in group 1 and only four accessions in smaller group 2. Group1 was further divided into subgroups i.e. subgroup A with 14 accessions and subgroup B with 6 accessions. Similar results of clustering of genotypes of common ancestor in single group were observed by Wang *et al.*, (2010). Zakova *et al.*, (2006) got similar results for spring barley accessions.

**Fig.1** Clustering of ICARDA accessions on the basis of nine agro-morphological data using SPSS v23 software program



**Fig.2** Clustering of ICARDA accessions on the basis of microsatellite markers using SPSS v23 software program



**Table.1** Estimates of genetic parameters for different quantitative and qualitative characters of barley at ICARDA- IRP, Amlaha, Madhya Pradesh, India

Parameter	PH	T/P	DH	S/P	SL	Sp/S	GN	TGW	Y/P
<b>Heritability</b>	9.454	41.203	41.122	7.556	74.135	75.405	62.204	24.331	24.331
<b>GCV</b>	4.492	20.213	20.08	3.53	31.111	31.749	18.6	19.784	19.784
<b>PCV</b>	14.608	31.489	31.314	12.841	36.132	36.562	23.584	40.107	40.107
<b>Genetic Advance</b>	2.157	3.253	3.221	0.156	30.667	30.936	15.147	2.07	10.349
<b>Genetic Advance value % means</b>	2.845	26.727	26.526	1.999	55.181	56.794	30.22	20.103	20.103

*PH* = Plant height, *T/P* = Tillers /plant, *DH* = Days to heading, *S/P* =Spikes per plant, *SL* = Spike Length, *Sp/S* = Spikelet per spike, *GN* = Grains per spike, *TSW* = 1000 grains weight, *Y/P* = Yield/ Plant, *GCV* = Genotypic Coefficient of Variance, *PCV*=Phenotypic Coefficient of Variance

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

Primer's name	Chr	Tm(c)	No.of Alleles	Allels size range(bp)	High frequency allele	% of high frequency allele	PIC
Bmag211	1H	58	4	156-176	166	37.50	0.52
ABG500a	1H	55	3	170-189	155	30.00	0.59
Bmag105	1H	60	2	100-108	100	37.50	0.47
Bmag213	1H	55	2	150-180	150-180	30.00	0.50
Scssr10477	1H	55	2	110-130	130	32.50	0.50
Bmag829	2H	55	3	170-189	180	32.50	0.59
HVM54	2H	55	3	110-159	130	30.00	0.59
Bmag749	2H	55	3	120-166	110	47.50	0.34
Bmac576	2H	53	3	100-160	149	27.50	0.64
EBmac640	2H	58	3	110-176	176	32.50	0.60
Bmac129	2H	58	4	100-150	145	27.50	0.68
ABC252	2H	58	3	200-250	250	45.00	0.47
ABG058	2H	60	3	280-330	330	30.00	0.62
HVM36	2H	55	5	100-150	120	27.50	0.72
Bmag877	3H	55	3	120-153	140-153	22.50	0.68
Bmag225	3H	58	4	110-162	140	37.50	0.50
EBmac705	3H	55	2	140-150	140-150	30.00	0.50
ABG471	3H	58	3	940-1000	980	45.00	0.27
ABG70	3H	58	3	400-490	490	35.00	0.55
Bmag0013	3H	58	2	140-155	140	35.00	0.49
HVM14	4H	55	2	120-158	158	45.00	0.38
ABG500b	4H	58	2	180-189	180-189	30.00	0.50
MWG634	4H	58	2	880-900	900	50.00	0.28
Bmac175	4H	58	2	145-155	155	35.00	0.49
HVM40	4H	55	5	130-170	140	17.50	0.59
ABC483	5H	58	2	500-510	510	37.50	0.47
Bmag812	5H	55	2	120-157	157	50.00	0.28
Bmag222	5H	58	3	140-179	160	27.50	0.64
ABC302	5H	58	3	980-1100	1100	45.00	0.41
GMS61	5H	60	2	300-310	310	45.00	0.38
Bmag387	5H	58	2	110-150	150	42.50	0.41
Bmag163	5H	55	3	110-150	150	27.50	0.62
Bmag760	5H	55	3	110-130	160	27.50	0.68
MWG684	6H	58	5	150-210	210	15.00	0.81
HVM67	6H	55	2	110-120	110	40.00	0.44
MWG798	6H	58	2	350-370	350	40.00	0.44
Bmac0040	6H	58	3	200-236	236	30.00	0.59
Bmac64	7H	58	2	130-155	155	47.50	0.35
Bmag273	7H	55	2	120-186	186	55.00	0.15
Bmag341	7H	55	3	140-200	158	37.50	0.54
Bmac162	7H	58	3	160-187	170	30.00	0.61
bmag110	7H	58	2	130-145	145	37.50	0.47
Bmac224	7H	55	2	166-176	176	45.00	0.38

*Tm* = Annealing Temperature, *PIC*= Polymorphism Information content.

Similar level of genetic similarity values were reported for Indian barley during UPGMA based clustering (Verma *et al.*, 2015 and Yadav *et al.*, 2015)

### **Phenotypic and molecular comparative analyses**

The mantel correspondance test (Mantel, 1967) was used to compare the molecular and morphological similarity matrices. Correlation between the distance coefficient was observed low ( $r = 0.01204$ ,  $p = 0.5798$ ). Both morphological and molecular dendrograms clustered the accessions into two major clusters and 1-2 minor groups.

Molecular dendrogram cluster the different accessions into same groups which are not in the same group while clustering have been done for agro-morphological variations Correlation between the distance coefficient was low ( $r = 0.01204$ ,  $p = 0.5798$ ) during mantel correspondance test for molecular and morphological similarity matrices. Although dendrograms clustered the accessions into major and minor groups.

As reported earlier, there is no need to be a necessarily positive correlation between morphological and molecular markers specially SSRs because of their genetic nature, as there are studies (Zhang *et al.*, 2010) revealing no or low correlation in this regard. Nevertheless, the genetic relationship observed using molecular markers may provide information on the history and biology of accessions or genotypes, but does not necessarily reflect what may be observed with respect to agro-morphological traits (Metais *et al.*, 2000). The present study's results were in agreement with finding of several authors in different crops like in wheat (Salem *et al.*, 2008); faba bean (Ammer *et al.*, 2015), maize (Beyene *et al.*, 2005) and barley (Amabile *et al.*, 2013 and Singh *et al.*, 2014). The knowledge about the genetic relationships of genotypes provides useful information to address breeding program and germplasm resource management. In present study,

accessions performance evaluated in Indian condition can be further used for introduction or hybridization in any breeding program for any specific purpose enriching gene pool of barley in Indian. Markers information used in conjunction with morphological data, classified genotypes better than classifications based on individual data set i.e. morphological or molecular data. Genotypes selected through selection index can be evaluated in other agro-climatic zones of India for further utilization.

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