

Genetic diversity analysis of indigenous and exotic germplasm of barley (*Hordeum vulgare* L.) and identification of trait specific superior accessions

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Article history

Received: 28 Sep., 2018

Revised : 22 Nov., 2018

Accepted: 04 Dec., 2018

Citation

Kaur V, Kumari J, Manju, SR Jacob and BS Panwar. 2018. Genetic diversity of indigenous and exotic germplasm of barley (*Hordeum vulgare* L.) and identification of trait specific superior accessions. *Wheat and Barley Research* 10(3):190-197. doi.org/10.25174/2249-4065/2018/83620

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Abstract

Barley is an important feed, food, malt and brew purpose crop in India. The new challenges for production in the era of climate change envisage involvement of diverse germplasm for further exploitation in breeding. Genotypic variation in 256 indigenous and exotic barley germplasm accessions was evaluated for nine quantitative and seven qualitative traits during two consecutive cropping years (2014–15 and 2015–16). Indigenous collections were assembled from seven different states of India representing four agro-ecological zones while exotic material comprised of 80 accessions from USA and Syria. The coefficient of variation ranged from 3.47–41.21% in Indian accessions to little bit higher (3.34–56.14%) in exotic germplasm suggesting exotic accessions were more diversity. The principal component analysis showed that four most informative components could describe 72.22% of total multivariate variance and cluster analysis divided all accessions into four clusters showing association between genetic diversity and geographical diversity. Spike and grain traits were contributing more to variability among the accessions and lesser to peduncle length, plant height and days to maturity. Further superior accessions namely IC0364040 (tall land race with more grains/spike), IC0036966 (early maturing), IC0041585 (tall, high yield), IC0398681 (long spikes, tall), hull-less exotic landraces EC0362267 (more spike triplets/spike, short plant height) and EC0481703 (long spike, high yield, two-rowed) were identified and than be utilization as donors in breeding programs for different agro-ecologies.

Keywords: Barley germplasm, characterisation, genetic diversity, promising donors

1. Introduction

Barley (*Hordeum vulgare* L; 2n=14) is one of the first domesticated cereals and is considered a sacred grain in India since ancient times. Once regarded as poor man's food and fodder cereal, barley has gained importance again owing to its increased use in multigrain blends, health tonics, malting and brewing industries. This crop is being cultivated globally and it successfully grows in diverse eco-geographical regions such as the arid Sahara,

Tibetan plateaus, Himalayan hills, and the tropical plains of India (Abebe *et al.*, 2010). It is an important source of feed, forage for livestock, and of food, and drink for humans. Barley is considered a hardy crop due to anthesis earlier than wheat to ensure pollination and grain filling before onset of severe stress, and also owing to more extensive root system (Lopez-Castaneda and Richards, 1994; Bingham and Bengough, 2003; Manschadi *et al.*,

2006). Thus, it is a better option for poor farmers to cope with the climate change, land degradation, poor rainfall, desertification and saline-alkaline soils as yields are generally considered to vary less under changing weather conditions than those of wheat and most other small grains (Cossani *et al.*, 2011; Newton *et al.*, 2011). In congruence with above said, Dawson and co-workers (2015) have highlighted barley as a resilient food crop for future nutritional security and a translational model for adaptation to climate change in a recent review on barley. Presently, barley is the fourth most important cereal crop worldwide (after maize, rice, and wheat) producing 141 MT (Metric tonnres) in 2016, while in India, it is cultivated on about 0.59 Mha area with production of 1.51MT and productivity of 2550 kg/ha (FAO, 2018). It is usually grown in the northern plains as well as northern hills mostly under rainfed or limited irrigation conditions; however recent surge in industrial demand for malting and brewing has transformed its cultivation into an optimally managed commercial crop particularly under contract farming in Punjab and Haryana. Preference of particular traits over others by breeders' has led to loss of specific alleles over time and thus creation of a narrow germplasm base. Therefore, to diversify parental material hitherto unknown sources of genetic diversity through evaluation of germplasm must be incorporated into plant breeding. UPOV and IPGRI (now known as Bioversity International) recommend morphological characterization

as criteria to characterize and identify accessions for the estimation of genetic diversity. Earlier, barley germplasm assembly from different regions of world viz. Ethiopia (Derbew *et al.*, 2013; Abebe *et al.*, 2010); Spain (Lasa *et al.*, 2001); North America (Mikel and Kolb, 2008); Sardinia (Papa *et al.*, 1998); India (Manjunatha *et al.*, 2007; Sarkar *et al.*, 2010, 2014); Jordan (Shakhatreh *et al.*, 2010); Slovakia (Žáková and Benková, 2004) and Bhutan (Konichi *et al.*, 1993) have been subjected to phenotypic diversity assessment studies. Assessment of the extent of genetic variability in germplasm collections is particularly useful for identification of trait specific superior genotypes. The present study was therefore, conducted to evaluate a set of 256 indigenous and exotic barley germplasm collections using a range of univariate to multivariate analysis methods to quantify genetic diversity, and to identify superior germplasm which may contribute as probable donors for future exploitation in selection and breeding.

2. Materials and methods

Plant material: In total, 256 barley germplasm accessions representing 176 indigenous collections (IC) and 80 exotic collections (EC) were studied. The IC were assembled from seven different states of India representing four agro-ecological regions of the country while the EC were from USA and Syria (Table 1).

Table 1. Details of barley germplasm accessions along with their initial sources/collection areas and biological status

	Country	Zone	State	Bio-status	No. of accessions		
Indigenous	India	Eastern Himalayan region (II)	Arunachal Pradesh	Landrace	3		
				Unknown	3		
				Sikkim	Landrace	5	
				Unknown	44		
			Assam	Landrace	1		
				Improved cultivar	1		
			Lower Gangetic Plains (III)	West Bengal	Landrace	3	
					Unknown	1	
			Middle Gangetic Plains (IV)	Bihar	Landrace	13	
					Improved cultivar	21	
					Unknown	17	
					Jharkhand	Landrace	8
						Unknown	11
			Central Plateau and Hills region (VIII)	Madhya Pradesh	Improved cultivar	15	
					Landrace	1	
Unknown	29						
Exotic	USA				78		
	Syria				2		
Total					256		

Field trials: Field trials were carried out in an Augmented Block Design (Federer, 1956) during the Rabi season consecutively for two years (2014–2015 and 2015–2016) at Issapur experimental farm of ICAR-National Bureau of Plant Genetic Resources, New Delhi located between 28°57' N, 76°84' E, altitude 218 m above sea level (asl) in south-west of Delhi, India. Issapur has semi-arid subtropical climate with average annual precipitation received around 400 mm. The soil of the farm varies from sandy loam to loamy sand with pH around 8. All the accessions were grown in three rows of 2 m row length, with a between-row spacing of 25 cm, and a within-row spacing of 10 cm in the 11 blocks. Each block had 23 accessions and four standard check varieties BH902, DL88, Jyoti and DWRB101, which were randomized in each block. Recommended agronomic practices were followed through various stages of crop growth. The data were recorded on 16 traits, of which 9 traits viz. days to 75% spike emergence (DAY_SPEM), days to 80% maturity (DAY_MAT), plant height (PLT_HGT), peduncle length (PED_LEN), spike length (SPIK_LEN), spikelet triplet groups (SPIKELET), grains number per spike (GRN_SPK), 100-grain weight (GRN_WT), and grain yield per metre row (GRN_YLD) were measured quantitatively, while another 7 traits viz. growth class, growth habit, spike row type, awn type, spike density, grain type and grain pericarp color were assessed qualitatively on different scales. All the traits were measured on randomly selected five plants for each accession as per minimal descriptors for barley (Mahajan *et al.*, 2000). The trait specific superior accessions identified in the present study were further validated during the year 2016-17 and 2017-18.

Statistical analysis: All the quantitative data were subjected to statistical analysis for mean, range, variance, correlation and principal component analysis using statistical software SAS (SAS version 9.3, 2009). As the error variances were homogenous; there was no need for data transformation and data was pooled to perform combined analysis. k-means clustering method was used to classify accessions in discrete clusters based on information from Hierarchical clustering. Combination of Principal components analysis (PCA) and cluster analysis approaches gave comprehensive information of characters which are critically contributing for genetic variability in barley accessions under study.

3. Result and discussions

Evaluation for morpho-phenological and agronomic traits: All the accessions under study were spring barley type. Semi spreading nature of growth habit (GRW_HAB) was

represented by majority of the germplasm (87%) while prostrate type (2%) and erect (11%) were less represented. Awned spikes were recorded in 247 accessions, while awnleted and sessile hoods were recorded in only 4 accessions in the entire assembly under study and only one accession EC492301 was recorded as awnless. Spike row (SPIK_ROW) in 95% of the barley accessions were six-rowed type in contrast to two-rowed observed in 5% of accessions. Spike density (SPK_DEN) recorded by measuring the length of rachis internode categorized germplasm into dense (37%), intermediate (42%) and lax (21%) types. A wide range of variation was recorded with respect to grain pericarp color (GRN_CLR) ranging from white, blue, black and red in some accessions, however white/yellow pericarp was dominant (87%). Hulled grains were more abundant (81%) while hull-less were represented in 19% accessions only. Thus, in the present study, the most common type of barley germplasm accessions were characterized by semi spreading nature of growth habit, six-rowed awned spikes with intermediate spike density, hulled white/yellow grains which is also in congruence with earlier reports by Manjunatha *et al.*, (2007) and Sarkar *et al.* (2010) for almost similar frequency distribution of above mentioned traits in barley germplasm. General statistical analysis showed wide range of phenotypic expression for different traits in the collection indicative of presence of good amount of variability (Table 2). The accessions varied widely in both time to heading and maturity. DAY_SPEM showed continuous variation from 77 to 107 days. DAY_MAT ranged continuously from 122 to 147 days, in the studied indigenous barley germplasm, while it was 117 to 142 days in exotic accessions. One hooded accession EC0667420 was recorded showing early maturity in 117 days compared to check BH902 which took 131 days to attain maturity. Mean PLT_HGT recorded was 90.48 cm in indigenous accessions which was slightly lesser (84.72 cm) in exotic landraces. Exotic material showed more variability (CV = 3.34-56.14%) in all quantitative traits except DAY_MAT and SPIKELETS when compared with indigenous assembly (CV = 3.47-41.21%). GRN_WT, an important yield character, varied extensively from 1.87g to 5.61g. It was notable that the characters with the highest variability (CV = 40.44%) was GRN_WT, a key component of yield and grain yield itself (CV = 56.14%) suggesting that GRN_WT is a key target for improvement. Thus, the variation for the different characters found in indigenous and exotic cultivars included in this study could be exploited and used in future barley improvement programs. Earlier morpho-phenological and agronomic quantitative traits have frequently been used for studying

Table 2. Range of variation for various agro-morphological traits in barley based on pooled average for two years (2014–15 and 2015–16)

Trait	Minimum	Maximum	Range	Mean	Std Dev	CV (%)	Variance	Std Error
Indigenous accessions (n=176)								
DAY_SPEM	77	107	30	93.41	3.24	3.47	10.52	0.24
DAY_MAT	121.5	147	25.5	131.57	5.17	3.93	26.74	0.39
PLT_HGT	67.42	114.58	47.17	90.48	8.45	9.34	71.47	0.64
PED_LEN	26.78	52.6	25.82	39.77	4.78	12.02	22.84	0.36
SPIK_LEN	4.92	10.42	5.5	7.76	0.92	11.89	0.85	0.07
GRN_SPK	18.67	68.33	49.67	41.27	8.55	20.72	73.12	0.64
SPIKELET	8	28.33	20.33	18.57	3.37	18.14	11.35	0.25
GRN_WT	1.87	5.61	3.74	4.16	1.16	27.88	1.34	0.09
GRN_YLD	100.63	432.5	331.88	264.85	109.14	41.21	11911.5	8.23
Exotic accessions (n=80)								
DAY_SPEM	77	106.5	29.5	98.08	6.74	6.87	45.45	0.75
DAY_MAT	117.4	142	24.6	131.19	4.39	3.34	19.24	0.49
PLT_HGT	61.83	107.07	45.23	84.72	8.67	10.23	75.13	0.97
PED_LEN	26.4	59.35	32.95	40.44	7.03	17.39	49.48	0.79
SPIK_LEN	5.93	11.57	5.63	8.04	1.12	13.88	1.25	0.12
GRN_SPK	16	59.5	43.5	36.17	11.14	30.8	124.06	1.25
SPIKELET	11.33	25.67	14.34	18.6	3.2	17.23	10.26	0.36
GRN_WT	1.09	5.59	4.5	3.54	1.43	40.44	2.05	0.16
GRN_YLD	100.63	504.17	403.54	183.68	103.12	56.14	10633.6	11.53

DAY_SPEM, days to spike emergence; DAY_MAT, days to physiological maturity; PLT_HGT, plant height (cm); PED_LEN, peduncle length (cm); SPIK_LEN, spike length (cm); GRN_SPK, number of grains per spike; SPIKELET, spikelet triplet groups; GRN_WT, hundred grain weight (g); GRN_YLD, grain yield per meter row (g); n, number of accessions.

genetic diversity in barley (Abebe *et al.*, 2010; Ahmad *et al.*, 2008; Assefa and Labuschagne, 2004; Fu, 2012; Lasa *et al.*, 2001; Manjunatha *et al.*, 2007; Papa *et al.*, 1998; Shakhathreh *et al.*, 2010) and reported variability for plant height, hundred-grain weight and grain yield in barley. Phenotypic correlation coefficients computed to study the association among nine quantitative traits are presented in Table 3. Plant height is an important

trait directly linked with the grain yield potential of the plant in addition to being an important fodder trait in barley. In our study, the Pearson correlation coefficient between plant height and grain yield was positive and significant ($r = 0.27$, $P < 0.01$). Ruzdik *et al.*, (2015) and Drikvand *et al.*, (2011) also observed significant and positive correlation between plant height and grain yield in barley. In addition, PLT_HGT was also significantly

Table 3. Correlation between different morpho-phenological and agronomic traits for 256 germplasm accessions of barley

Traits (n=256)	DAY_SPEM	DAY_MAT	PED_LEN	PLT_HGT	SPIK_LEN	SPIKELET	GRN_SPK	GRN_WT
DAY_MAT	0.06							
PED_LEN	0.06	-0.25**						
PLT_HGT	-0.09	-0.19**	0.29**					
SPIK_LEN	0.22**	-0.07	0.03	0.08				
SPIKELET	0.01	-0.28**	0.03	0.14*	0.26**			
GRN_SPK	-0.02	-0.12	0.04	0.22**	0.31**	0.22**		
GRN_WT	-0.21**	-0.14*	-0.11	-0.03	0.15*	-0.01	0.60**	
GRN_YLD	-0.38**	-0.13*	-0.03	0.27**	0.04	-0.06	0.25**	0.28**

* and ** indicate correlation significance at the P0.05 and P0.01 levels of probability, respectively.

DAY_SPEM, days to spike emergence; DAY_MAT, days to physiological maturity; PED_LEN, peduncle length; PLT_HGT, plant height; SPIK_LEN, spike length; SPIKELET, spikelet triplet groups; GRN_SPK, number of grains per spike; GRN_WT, hundred grain weight; GRN_YLD, grain yield per meter row.

and positively correlated with other yield components such as GRN_SPK ($r = 0.22, P0.01$) and SPIKELETS ($r = 0.14, P0.05$). DAY_SPEM showed positive correlation ($r = 0.22, P0.01$) with SPIK_LEN as also reported by Mohtashami (2015) and Al-Tabbal and Al-Fraihat (2012) who found a positive and significant correlation between the spike length and days to heading. DAY_SPEM and DAY_MAT had negative correlation with GRN_WT and GRN_YLD. Our correlation results corroborated the studies by Al-Tabbal and Al-Fraihat (2012) and Sarkar *et al.* (2014) who also reported negative correlation between DAY_SPEM, GRN_WT and GRN_YLD and suggested early spike emergence may lead to more filled bold grains and thus yield in barley.

Clustering pattern: The clustering pattern based on nine quantitative variables revealed that the majority of the accessions grouped into four major clusters comprising 100 accessions in cluster I, 24 accessions in cluster II, followed by 62 accessions in cluster III and 70 accessions in cluster IV. Means of quantitative variables based on nine quantitative traits in different clusters of barley germplasm accessions are presented in Table 4.

Table 4. Means of quantitative variables in different clusters of barley germplasm accessions

Traits*	Cluster 1	Cluster 2	Cluster 3	Cluster 4
DAY_SPEM	97.52	92.41	93.03	93.66
DAY_MAT	132.74	130.73	130.61	130.6
GRN_SPK	36.58	41.09	44.09	39.68
SPIKELET	18.54	18.51	18.67	18.61
SPIK_LEN	7.83	8	7.83	7.86
PED_LEN	39.92	39.3	39.98	40.36
PLT_HGT	85.55	89.93	92.13	89.87
GRN_WT	3.66	4.96	4.47	4.38
GRN_YLD	129.35	468.04	341.8	229.41
No of accessions in each cluster	100	24	62	70
Agro-climatic zone	III, VIII	-	II, IV	II, IV

*DAY_SPEM, days to spike emergence; DAY_MAT, days to physiological maturity; GRN_SPK, number of grains per spike; SPIKELET, spikelet triplet groups; SPIK_LEN, spike length (cm); PED_LEN, peduncle length (cm); PLT_HGT, plant height (cm); GRN_WT, hundred grain weight (g); GRN_YLD, grain yield per meter row (g).

The resulting four clusters distinguished accessions from different geographical areas in the region but failed to separate either naked from covered or two-row from six-row barley. Majority of the accessions from Madhya Pradesh (MP) and exotic accessions appeared under cluster I and IV, while most of the accessions from Sikkim and Bihar grouped under clusters III and IV. Largely the accessions from Eastern Himalayan region (zone II) and

Middle Gangetic Plains (IV) agro-climatic zones for barley were grouped under two clusters (III and IV) while Lower Gangetic Plains (III) and Central Plateau and Hills region (VIII) grouped under cluster I. Thus, a fair association between genetic diversity and geographical diversity was revealed. Our results are in congruence with studies by Abebe *et al.*, (2010) and Shafaeddin (2002) who reported fair relationship between genetic and geographical classification among the origin of samples. However, our clustering pattern doesn't corroborate the studies by Rabbani *et al.*, (1998), Shekhawat *et al.*, (2001) and Mittal *et al.*, (2010) who reported that geographical diversity may not necessarily reflect genetic diversity and suggested genetic similarity, environmental influence, selection for specific traits and exchange of material across sites as most probable reasons for grouping of same geographical regions in different clusters.

Principal Component Analysis (PCA): The variation studied through PCA revealed that four most informative principal components (PCs) having greater than one Eigenvalues contributed 72.22% of the total multivariate variation present in the studied germplasm. A quarter of the total variance was accounted for in the first axis, and $\approx 19\%$ in the second, confirming the independence of some characters as represented in the genotype-biplot based on first two PCs (Fig. 1).

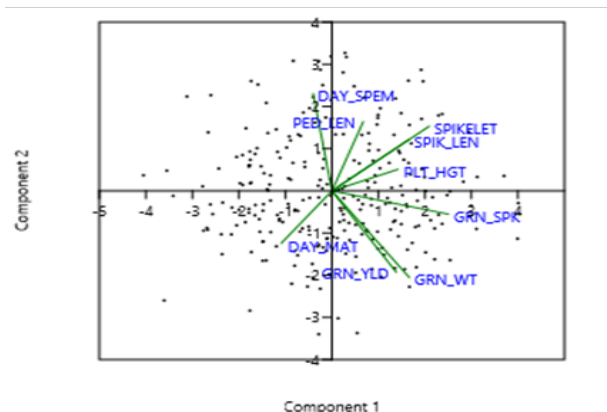


Fig. 1 Scatter plot different morpho-agronomic variables loaded on PC1 and PC2 DAY_SPEM, days to spike emergence; DAY_MAT, days to physiological maturity; PED_LEN, peduncle length; PLT_HGT, plant height; SPIK_LEN, spike length; SPIKELET, spikelet triplet groups; GRN_SPK, number of grains per spike; GRN_WT, hundred grain weight; GRN_YLD, grain yield per meter row.

The traits, which contributed more positively to PC1 were GRN_SPK, SPIK_LEN and SPIKELET, while GRN_WT and GRN_YLD contributed to PC2. Both positive (e.g. DAY_SPEM, PLT_HGT, PED_LEN, SPIK_LEN and SPIKELET) and negative (DAY_MAT, GRN_WT and GRN_YLD) associations were found in PCA. In general, PC1 and PC2 were mainly attributed to spike and grain traits, while PC3 and PC4 had greater weightage

for PLT_HGT, PED_LEN and DAY_MAT. High contribution of above mentioned traits was also reported by Manjunatha *et al.* (2007) and Ebrahim *et al.* (2015) while studying diversity analysis in barley germplasm. PC3 and PC4 explained ≈ 17 and 11% variation, respectively. The remaining variation (27.78%) was contributed by other five PCs (PC5–PC9). PCA suggests that germplasm accessions could be distinguished by characters associated with PCs having more Eigen values and vectors associated with them as also reported by Johnson and Wichern (2002). Thus, it is evident that pattern of variability as revealed by PCA plot was useful to identify distinct accessions apart from a group of morphologically identical overlapping ones.

Trait specific superior germplasm: The present study also entrusted to identify trait specific superior accessions which may serve as probable donors for utilization in barley breeding program.

Table 5. Trait specific superior accessions identified in barley germplasm along with their initial sources/collection areas and biological status

Trait	Promising donor	Area of collection	Bio-status
DAY_SPEM<80 days	IC0148374	Madhya Pradesh	Improved cultivar
	EC0362267N,R	USA	Landrace
DAY_MAT <125days	IC0036944N	Sikkim	Unknown
	IC0036973	Sikkim	Unknown
	IC0036966B	Sikkim	Unknown
	IC0036980R	Sikkim	Landrace
PLT_HGT <70cm	EC0667420H	Syria	Breeding line
	EC0362267N,R	USA	Landrace
	EC0481707N	USA	Landrace
	EC0362162N	USA	Landrace
PLT_HGT >110cm	IC0041585	Sikkim	Unknown
	IC0364040N,B	Sikkim	Landrace
	IC0398681	Bihar	Landrace
PED_LEN >50cm	IC0036975R	Sikkim	Unknown
	EC0050666	USA	Landrace
	EC0481708N,R	USA	Landrace
SPIK_LEN >10cm	IC0036979	Sikkim	Landrace
	IC0398681	Bihar	Landrace
	EC0481703*N	USA	Landrace
GRN_SPK >60	IC0036966B	Sikkim	Unknown
	IC0041576	Sikkim	Unknown
	IC0364040N,B	Sikkim	Landrace
SPIKELET >25	IC0041576	Sikkim	Unknown
	IC0036966B	Sikkim	Unknown
	EC0362267N,R	USA	Landrace
GRN_WT >5.5g	IC0036904	Unknown	Landrace
	IC0533035	Haryana	Landrace
	IC0397005	Rajasthan	Landrace
GRN_YLD >400g	IC0041585	Sikkim	Unknown
	IC0361397	Bihar	Improved cultivar
	EC0481703*N	USA	Landrace

*Two-row type; N=naked (hull-less); H= hooded barley; R= red pericarp color; B= light blue color; remaining all are six-row type, hulled with white pericarp). Accessions indicated in bold letters are promising for more than one trait. DAY_SPEM, days to spike emergence; DAY_MAT, days to physiological maturity; PLT_HGT, plant height; PED_LEN, peduncle length; SPIK_LEN, spike length; GRN_SPK, number of grains per spike; SPIKELET, spikelet triplet groups; GRN_WT, hundred grain weight; GRN_YLD, grain yield per meter row.

Table 5 enlists these promising accessions for different traits along with their area of collection and biological status. Tribal dominated belts of north eastern states of India (Eastern Himalayan zone) are rich in local variability of barley (Arora, 1988). In our study also, many important traits such as early maturity, more number of grains per spike, tall plant height and 100-grain weight are reflected in germplasm from Sikkim, indicating the diversity rich material available there. It is evident that most of the enlisted germplasm accessions are either landraces of USA or from Sikkim falling under cereal diversity rich Eastern Himalayan region (zone II, Table 1). For traits GRN_SPK, DAY_MAT, SPIKELETS; Sikkim accessions were superior while for short PLT_HGT, GRN_YLD, SPIK_LEN, GRN_WT mostly exotic accessions performed better. Few accessions were found to be superior for more than one trait e.g. IC0364040 (hull-less tall landrace with more grains per spike), IC0036966 (early maturity and more grain number per spike) and IC0041585 (tall and high yield) all three from Sikkim and one landrace from Bihar (IC0398681) for long spikes and tallness. This indicates possibility for effective trait mining in this zone and utilizing collections for the trait specific germplasm identification through statistical approaches like Focused Identification of Germplasm Strategy (FIGS). Hooded barley accession EC0667420 (early maturity), hull-less EC0362267 (more spike triplets per spike, dwarf, red pericarp) and EC0481703 (long spike, high yield, two-rowed) were found superior among exotic accessions.

In conclusion, the results of screening a diverse collection of 256 barley accessions has been a contribution to increase the knowledge about the barley germplasm variability and also led to identification of superior genotypes with promising traits. Analysis of diversity pattern among accessions from different regions for quantitative traits revealed that accessions from Sikkim possessed early spike emergence, more spike triplets per spike, more number of grains per spike and early maturity. Exotic material showed better performance for greater spike length, early heading, 100-grain weight and short plant height. The characters, such as early heading, taller plants, and early maturity are likely to be of specific advantage in dry conditions as days to heading and days to maturity represent the best compromise to escape terminal drought. The information generated in this type of studies can be utilized in breeding program for yield as well as for other characters, as parents in the crossing program to introduce additional desirable characters in an adapted genetic background.

Acknowledgement

Authors acknowledge use of research facilities available at ICAR-NBPGR. Authors also express sincere thanks to all partners who helped in the exploration, collection and conservation of barley germplasm.

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