Journal of Cereal Research
13(1): 79-88
Research Article
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Identification of promising barley genotypes based on morphological genetic diversity

Yogender Kumar*, Krishan Dutt Sehrawat, Jogendra Singh[1] and Sachin Shehrawat

Wheat and Barley Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar-125004 (Haryana), India
[1]ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, India

Article history:
Received: 26 Dec., 2020
Revised: 27 Feb., 2021
Accepted: 23 March, 2021

Citation:
Kumar Y, KD Sehrawat, J Singh and S Shehrawat. 2021. Identification of promising barley genotypes based on morphological genetic diversity. *Journal of Cereal Research* ***13(1):*** *79-88.* http://doi.org/10.25174/2582-2675/2021/108051

****Corresponding author:***
E-mail: yogenderkgulia@gmail.com

Abstract

Estimation of genetic diversity in a crop species provides a basis for devising future strategies for their conservation and sustainable use in crop improvement. Genotypic variation in 72 barley genotypes was studied for ten morphological traits during two consecutive cropping years (2015-16 and 2016-17) at Chaudhary Charan Singh Haryana Agricultural University, Hisar. Coefficient of variation attributed to genotypic diversity was recorded highest for grain yield (26.16 %) followed by inclination angle (20.0 %), internode length (13.11 %) and tillers per meter (13.07 %). However, days to maturity (2.69 %) contributed less to diversity with lower coefficient of variation. The principal component analysis revealed that first four most informative components could explain about 71.0 % of total variation present in the studied genotypes. Hierarchical cluster analysis clubbed all the barley genotypes into eight clusters. Clusters III and V being the largest one with 14 genotypes each and with one genotype. Cluster I was the smallest. Among all, maximum distance was displayed by clusters I and VI, however, highest diversity was exhibited by cluster V. The genotypes namely MGL 21 (early heading), MGL 38 (early maturing), MGL 47 (short plant height with high tillering), MGL 12 (high culm thickness), MGL 117 (long internodes), MGL 15 (long spikes) and MBGSN 145 (high grain weight, high yield, lodging resistant) were identified as most diverse genotypes. Cluster I, IV and VII portrayed better performance for most of the traits studied. Genotypes from these groups could be utilized as donors in breeding programs for different agro-ecologies.

Key words: Barley, cluster analysis, diversity, morphological traits, principal component, promising donors

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the ancient domesticated cereal crops, currently ranking fourth after rice, wheat and maize in the world production (USDA, 2020). It is a true diploid (2n=14) species which belongs to the genus *Hordeum* of family *Poaceae* and tribe *Triticeae.* Barley is a nutrient rich cereal and occupies an area of 0.62 million hectare producing 1.59 million tonnes grain with productivity of 25.73 q/ha (Anonymous, 2020). It was cultivated on 12,200 hectares with a production of 44,000 tons in Haryana and ranked second in average productivity (36.07 q/ha) after Punjab (37.67 q/ha) during 2019-20 (ICAR-IIWBR, 2020). Barley cultivation requires less input in the form of fertilizer, irrigation and insecticides, and it has potential to grow under drought and saline conditions. Owing to their features it has been traditionally considered as poor man's crop throughout the world especially for people dependant on subsistence farming. Barley has gained importance owing to its increased use in multigrain blends, health tonics, malting and brewing industries (Kaur *et al.*, 2018).

Further, self pollination, diploid nature, short cell life, ease of hybridization and existence of genetic variation among wild and cultivated species are the factors that makes barley a model experimental system for various researches (Kumar *et al.,* 2014).

Genetic diversity is the base of plant breeding, and is one of the important components of stability of biological systems (Khajavi *et al.*, 2014). Thorough knowledge about the existing genetic diversity in available germplasm, its proper management and utilization is prerequisite and key factors for selection of the parents with diverse genetic background and to make crop improvement more efficient. Determining the level of variation within and among breeding populations is an essential step towards conserving genetic resources and developing future strategies. Information on the nature and degree of divergence among the genotypes helps the plant breeders in identification of trait specific superior donors for initiating targeted hybridization programme, as heterotic expression is believed to be associated with genetic divergence among the parents (Ramanujam *et al.*, 1974). Therefore, to diversify the parental material, thorough evaluation of germplasm must be incorporated into plant breeding programmes. For a long time, morphological traits have remained the means of studying genetic variations in plant species. The International Union for the Protection of New Varieties of Plants and International Plant Genetic Resources Institute (now known as Bioversity International) also recommend morphological characterization as criteria to identify accessions for the estimation of genetic diversity (Kaur *et al.*, 2018). Some studies on genetic diversity of barley have focused on importance of morphological and quantitative traits (Manjunatha *et al.*, 2007; Shakhatrech *et al.*, 2010; Mekonnon *et al.*, 2014; Yadav *et al.*, 2015b; Patial *et al.*, 2016; Grewal and Kaur, 2018). Several approaches are available to assay genetic diversity. Often Principal component analysis (PCA) and cluster analysis (Peeters and Martinelli, 1989) have been used to reduce large number of germplasm lines and to identify the important characters to be used in a fresh breeding programme with a greater degree of reliance.

The present study was, therefore, designed with 72 barley genotypes to quantify genetic diversity, and to identify trait specific promising genotypes which may contribute as probable donors for future exploitation in combination breeding.

2. Materials and Methods

Field experiment with 72 barley genotypes (Table 1) representing both 2-row (21) and 6-row (51) types was carried out at Experimental Farm, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar which is located in the semi-arid subtropics at 29°10' N latitude and 75°46' E longitude with an altitude of 215.2 m AMSL. The soils of the farm are predominantly sandy loam type. It received a total of 33.4 and 49.7 mm rainfall with mean monthly minimum and maximum temperatures of 10.8°C, 26.9°C and 10.5°C, 27.3°C during the crop seasons 2015-16 and 2016-17, respectively.

The experiment was laid out in an augmented design with 5 blocks. Each block contained 13 different genotypes along with 7 checks namely, DWRUB 52, DWRB 101, RD 2552, RD 2715, RD 2035, BH 946 and BH 902, repeated in each block every after 13th entry. Each genotype was grown in four rows of 2.5 m length with 23 cm spacing between rows. The experiment was conducted for two consecutive cropping seasons *i.e.*, *rabi* 2015-16 and 2016-17. The recommended cultural and agronomic practices were followed to raise the crop. A total of 10 morphological traits *viz*., days to heading, days to maturity, plant height (cm), culm thickness (mm), internode length (cm), inclination angle, tillers per meter, spike length (cm), 1000 grain weight (g) and grain yield per plot (g) were recorded at appropriate growth stages of the crop. Culm thickness and internode length were recorded on the 2nd basal internode with the help of vernier caliper and measuring scale, respectively. Inclination angle was recorded in order to measure the degree and intensity of lodging with the help of protractor. The observations were recorded on five random plants of each genotype, except for days to heading, maturity, 1000 grain weight and grain yield, which were recorded at plot level.

All the quantitative data pooled over two years (2015-16 and 2016-17) were subjected to statistical analysis for mean, range, coefficient of variation (CV), principal component and cluster analysis using statistical software SPSS (SPSS Statistics v. 19.0). The principal component analysis (PCA) was computed to reduce the number of variables into a few correlated components that can explain much of the variability. It was performed using

the correlation matrix to define the pattern of variation in the experimental material based on the mean of metric traits and to identify traits that load the most in explaining the observed variability. To retain the number of principal components, Kaiser's (1958) suggestion of dropping those principal components of correlation matrix with eigen roots less than one, was followed. Principal factor analysis was carried out using principal component method, which does not require assumption of multivariate normal distribution of population (Jaiswal, 2000). The factor axes were rotated using varimax method of orthogonal rotation (Kaiser, 1958) which is the most popular method and correspond to spreading out of the squares of loading on each factor as much as possible. It made possible to obtain groups of large and negligible coefficients in different columns of the rotated factor loading.

Genotypes were clustered using the method of average linkage between groups, often called UPGMA (unweighted paired group method using arithmetic averages) as it is suggested to be best and most commonly used method (Romesburg, 1990). In the present study, proximity matrix (City Block) was used to find out the relative distances between and within different clusters. Multivariate cluster analysis is very useful method in interpreting the results of agricultural experiments (Klikocka and Tatarczak, 2015).

Table 1. Barley genotypes used in the study

Genotypes	Row Type	Source of collection	Genotypes	Row Type	Source of collection	Genotypes	Row Type	Source of collection
MBGSN-145	2	ICARDA	MGL-36	6	ICARDA, Morocco	MGL-71	2	ICARDA, Morocco
MBGSN-147	6	ICARDA	MGL-37	2	ICARDA, Morocco	MGL-73	6	ICARDA, Morocco
NDB 1020-A	6	ND University, Faizabad	MGL-38	6	ICARDA, Morocco	MGL-75	6	ICARDA, Morocco
NDB 1040-A	6	ND University, Faizabad	MGL-39	6	ICARDA, Morocco	MGL-81	2	ICARDA, Morocco
RD 2902	6	RARI, Durgapura	MGL-40	2	ICARDA, Morocco	MGL-88	6	ICARDA, Morocco
RD 2905	6	RARI, Durgapura	MGL-41	6	ICARDA, Morocco	MGL-97	6	ICARDA, Morocco
RD 2911	6	RARI, Durgapura	MGL-42	6	ICARDA, Morocco	MGL-104	6	ICARDA, Morocco
RD 2912	6	RARI, Durgapura	MGL-43	2	ICARDA, Morocco	MGL-105	6	ICARDA, Morocco
MGL-5	6	ICARDA, Morocco	MGL-44	6	ICARDA, Morocco	MGL-106	2	ICARDA, Morocco
MGL-6	6	ICARDA, Morocco	MGL-45	6	ICARDA, Morocco	MGL-117	6	ICARDA, Morocco
MGL-10	2	ICARDA, Morocco	MGL-46	6	ICARDA, Morocco	MGL-127	6	ICARDA, Morocco
MGL-12	6	ICARDA, Morocco	MGL-47	6	ICARDA, Morocco	MGL-128	2	ICARDA, Morocco
MGL-14	2	ICARDA, Morocco	MGL-48	6	ICARDA, Morocco	MGL-129	6	ICARDA, Morocco
MGL-15	6	ICARDA, Morocco	MGL-51	6	ICARDA, Morocco	MGL-133	6	ICARDA, Morocco
MGL-17	2	ICARDA, Morocco	MGL-57	6	ICARDA, Morocco	MGL-159	2	ICARDA, Morocco

MGL-20	6	ICARDA, Morocco	MGL-58	6	ICARDA, Morocco	MGL-174	6	ICARDA, Morocco
MGL-21	6	ICARDA, Morocco	MGL-59	2	ICARDA, Morocco	MGL-186	2	ICARDA, Morocco
MGL-22	6	ICARDA, Morocco	MGL-60	2	ICARDA, Morocco	RD 2552	6	RARI, Durgapura
MGL-23	6	ICARDA, Morocco	MGL-61	6	ICARDA, Morocco	DWRUB 52	2	ICARDA, Morocco
MGL-24	6	ICARDA, Morocco	MGL-62	2	ICARDA, Morocco	DWRB 101	2	IIWBR, Karnal
MGL-28	6	ICARDA, Morocco	MGL-64	6	ICARDA, Morocco	RD 2715	6	RARI, Durgapura
MGL-30	6	ICARDA, Morocco	MGL-68	2	ICARDA, Morocco	BH 902	6	CCS HAU, Hisar
MGL-34	6	ICARDA, Morocco	MGL-69	2	ICARDA, Morocco	BH 946	6	CCS HAU, Hisar
MGL-35	6	ICARDA, Morocco	MGL-70	2	ICARDA, Morocco	RD 2035	6	RARI, Durgapura

3. Results and Discussion

The descriptive statistics and PCA of 10 traits as depicted in Table 2 showed high level of variation in barley genotypes. The genotypes varied widely for both days to heading (83-109 days) and maturity (121-141 days) with a general mean of 94.10 and 131.86, respectively. The general mean for plant height was 96.40 cm which ranged from 70 to 113 cm. Culm thickness was in the range of 1.30 to 2.70 mm with a general mean of 1.88 mm. The mean internode length recorded was 14.57 cm, varied widely from 8.90 to 18.90 cm. The trait inclination angle as recorded to test the genotypes for lodging showed a mean of 75.18. The lowest and highest tillers per meter among the genotypes recorded were 90 and 146, respectively. Spike length exhibited a range of 4.20 cm. Thousand grain weight, an important yield trait, varied widely from 27.40 to 52.90 g. The general mean for grain yield per plot was 601.24 g, showed variation from 307 g to 912 g. It was notable that the trait with highest variability was grain yield (26.16 %) followed by inclination angle (20.00 %), internode length (13.11 %) and tillers per meter (13.07 %). Among all variables, for days to maturity the lowest coefficient of variation (2.69 %) was recorded. Our results are in congruence with studies carried out by Kumar *et al.* (2018a); Saroei *et al.* (2017); Amezrou *et al.* (2018) and Yadav *et al.* (2015a) for wide range of different quantitative traits in barley.

The variation studied through PCA, using the 10 quantitative traits revealed that the first four most informative components with eigen value ≥ 1.00 accounted for about 71 % of the total variation present in the studied genotypes (Table 2). Remaining components contributed little amount to the total variation, suggesting these components are of lesser practical value in barley improvement. The first principal component (PC1) accounted for 19.13 % of the total variance with days to maturity, heading, spike length and inclination angle, having contributing factor loadings of 0.775, 0.729, 0.712 and 0.420, respectively. The second PC explained 18.39 % of the total variation and plant height (0.813) and internode length (0.747) had the highest positive loading. The third PC contributed 17.15 % of the total variation and had high contributing factor loadings from tillers per meter (0.877), while culm thickness had the largest negative loading (-0.835). The fourth component that explained 16.33 % of the total variation was associated with high positive loadings of grain yield (0.787) and 1000 grain weight (0.759). The results of the present study can be used for developing well defined approach based on evaluation and characterization of genetic variation in barley and can be utilized in various breeding programmes. Our results substantiated the earlier studies (Kumar *et al.,* 2018b) reporting 81.37 % of cumulative variability explained by five principal components. The relative contribution and

importance of various traits to the total variability has also been reported by Manjunatha *et al.* (2007), Rahal-Bouziane *et al.* (2015), Dyulgerova *et al.* (2016), Amezrou *et al.* (2018) and Saroei *et al.* (2017) in barley. Mekonnon *et al.* (2014) also showed the presence of high genetic variation among barley genotypes based on principal component analysis.

Table 2. Descriptive statistics and principal component analysis (PCA) of studied morphological traits in barley

Traits	Mean±SEM	Maximum	Minimum	Range	CV (%)	PC 1	PC 2	PC 3	PC 4
Days to heading	94.10±0.70	109	83	26	6.27	0.729	-0.370	0.186	-0.383
Days to maturity	131.86±0.42	141	121	20	2.69	0.775	-0.311	0.105	-0.163
Plant height	96.40±0.86	113	70	43	7.59	0.094	0.813	-0.256	0.239
Culm thickness	1.88±0.03	2.70	1.30	1.40	11.88	-0.085	0.103	-0.835	0.103
Internode length	14.57±0.23	18.90	8.90	10.00	13.11	-0.165	0.747	-0.008	0.019
Inclination angle	75.18±1.77	90	43	47	20.00	0.420	-0.507	-0.164	0.379
Tillers per meter	112.40±1.73	146	90	56	13.07	0.181	-0.033	0.877	0.191
Spike length	8.03±0.12	10.40	6.20	4.20	12.35	0.712	0.317	0.158	0.128
1000 grain weight	38.88±0.48	52.90	27.40	25.50	10.52	-0.008	0.016	0.256	0.759
Grain yield	601.24±18.54	912	307	605	26.16	-0.148	0.130	-0.145	0.787
Eigen values						2.885	1.693	1.285	1.238
Per cent variability						19.132	18.391	17.145	16.328
Cumulative variability %						19.132	37.523	54.668	71.000

CV: Coefficient of variation; PC: Principal component

The hierarchical cluster analysis based on 10 quantitative traits grouped all the genotypes into eight clusters (Table 3). The cluster membership profile recognized cluster III and V as largest one with 14 genotypes each, followed by cluster II (12), VIII (11), IV (9), VII (8) and cluster VI (3), while the cluster I being smallest with one genotype only. However, the resulting clusters failed to distinguish the two-row from six-row barley. Kumar *et al.* (2020) used hierarchical cluster analysis to classify 87 barley genotypes based on 10 qualitative traits and also identified the promising genotypes for the future use in barley breeding. The association among the different genotypes is presented in the form of dendrogram (*Fig.* 1) prepared using rescaled distances. The dendrogram showed the relative magnitude of resemblance among the different clusters. The intra and inter-cluster distances were also calculated using proximity matrix (city block). The inter-cluster distance was higher than the intra-cluster, indicating wide genetic diversity among the genotypes. The maximum intra-cluster distance was recorded for cluster V followed by cluster II and cluster IV, implies that the genotypes in these clusters were relatively more diverse than the other clusters. Similarly, minimum intra-cluster distance was observed in cluster I since it contained only one genotype. It was reported that genotypes within the cluster with high degree of divergence would produce more desirable breeding materials for achieving maximum genetic advance (Singh *et al.,* 2014). The results also revealed that cluster I is the most distantly placed from cluster VI which is the maximum among all cluster combinations, followed by clusters VI and VII. However, cluster III is most closely placed to cluster IV which is minimum distance among inter cluster distances. It is well recognized that greater the distance between clusters, wider the genetic diversity would be between the genotypes. Therefore, highly divergent genotypes

would produce a broad spectrum of segregation in the subsequent generations enabling further selection and improvement. Ebrahim *et al.* (2015), Sarkar *et al.* (2014), Yadav *et al.* (2015b), Kumar *et al.* (2016) and Hailu *et al.* (2016) also studied and reported the existence of wide genetic diversity in barley.

Table 3. Distribution of 72 barley genotypes into different clusters

Clusters	No. of genotypes	Genotypes
I	1	MBGSN-145 (1)
II	12	MBGSN-147 (2), RD 2902 (5), MGL-23 (19), MGL-45 (34), MGL-48 (37), MGL-51 (38), MGL-57 (39), MGL-60 (42), MGL-97 (54), MGL-128 (60), MGL-159 (63), MGL-186 (65)
III	14	NDB 1020-A (3), NDB 1040-A (4), RD 2905 (6), MGL-21 (17), MGL-22 (18), MGL-24 (20), MGL-35 (24), MGL-38 (27), MGL-42 (31), MGL-44 (33), MGL-88 (53), MGL-104 (55), MGL-129 (61), MGL-133 (62)
IV	9	RD 2911(7), MGL-17 (15), MGL-20 (16), MGL-43 (32), MGL-46 (35), MGL-59 (41), MGL-68 (46), MGL-69 (47),DWRUB 52 (67)
V	14	RD 2912 (8), MGL-5 (9), MGL-10 (11), MGL-12 (12), MGL-14 (13), MGL-28 (21), MGL-34 (23), MGL-36 (25),MGL-39 (28),MGL-40 (29), MGL-41 (30), MGL-47 (36), MGL-70 (48), MGL-71 (49)
VI	3	MGL-6 (10), MGL-30 (22), MGL-37 (26)
VII	8	MGL-15 (14), MGL-58 (40), MGL-105 (56), MGL-117 (58), MGL-174 (64), RD 2552 (66), DWRB 101 (68),BH 946 (71)
VIII	11	MGL-61 (43), MGL-62 (44), MGL-64 (45),MGL-73 (50), MGL-75 (51), MGL-81 (52), MGL-106 (57),MGL-127 (59), RD 2715 (69), BH 902 (70), RD 2035 (72)

*Values in parenthesis indicates serial number of genotypes

Most of the clusters showed considerable differences in the mean values for the characters under study (Table 4). Cluster I exhibited minimum number of days to heading and shortest plant height; and also possessed highest inclination angle, tillers per meter, 1000 grain weight and grain yield. Cluster II was characterized by longest spikes. Cluster IV was demonstrated early maturity, longest internode, high tillers per meter, long spikes and moderately high 1000 grain weight. Cluster VI illustrated with minimum inclination angle, indicating the genotypes of cluster that are more prone to lodging and had weak strength of stem. The genotypes of cluster VII showed early heading, high culm thickness, long internodes with high grain yield. Several genetic diversity studies have been conducted on barley based on quantitative traits for selecting genetically diverse parents for hybridization (Sharma *et al.*, 2014; Dyulgerova *et al.*, 2016; Sarkar *et al.*, 2014).

Table 4. Means of morphological variables in different clusters of barley genotypes

Characters	Clusters							
	I	II	III	IV	V	VI	VII	VIII
Days to heading	87	97	91	92	98	99	90	93
Days to maturity	131	132	130	129	133	133	131	132
Plant height (cm)	86	93	98	97	93	91	101	100
Culm thickness (mm)	1.7	1.9	1.9	1.8	1.8	1.8	1.9	1.9
Internode length (cm)	10.9	14.8	14.4	15.5	13.9	13.2	15.4	14.8
Inclination angle	90	78	74	72	74	61	77	78
Tillers per meter	127	112	105	126	114	112	106	113
Spike length (cm)	7.9	8.3	7.8	8.2	8.1	7.8	7.8	8.0
1000 grain weight (g)	52.9	37.4	37.0	41.5	37.8	38.0	40.1	40.0
Grain yield (g/plot)	912	517	597	663	410	324	852	755

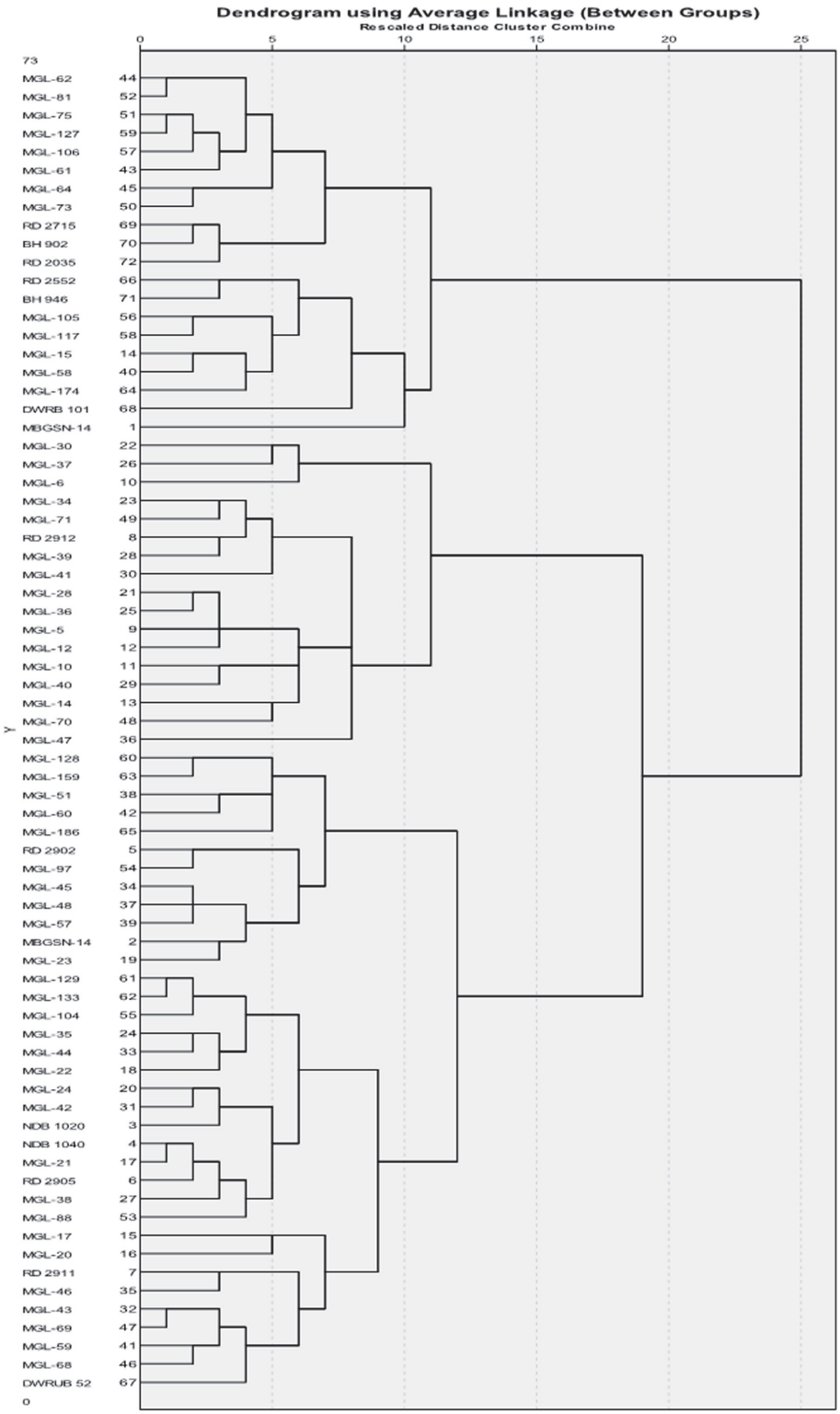

Fig. 1: Dendrogram portraying genetic diversity in barley genotypes based on morphological traits

Most diverse and promising genotypes with desirable traits selected from different clusters are represented in Table 5. The genotypes namely MGL 21 (early heading), MGL 38 (early maturing), MGL 47 (short plant height with high tillering), MGL 12 (high culm thickness), MGL 117 (long internodes), MGL 15 (long spikes), MBGSN 145 (high grain weight, high yield, lodging resistant) were identified as most diverse genotypes. From this study, it can be concluded that clusters I, IV and VII might be considered desirable for selecting genotypes which may be used as promising parents for hybridization.

Table 5. Trait specific promising barley genotypes in different clusters

Trait	Promising donors
Days to heading (≤ 85 Days)	NDB 1020-A, NDB 1040-A, RD 2902, MGL 21, MGL 38
Days to maturity (≤125 Days)	NDB 1040-A, RD 2902, MGL 17, MGL 21, MGL 38
Plant height (≤85 cm)	MBGSN 147, MGL 23, MGL 30, MGL 47, MGL 60, MGL 186
Culm thickness (≥2.2 mm)	RD 2902, MGL 12, MGL 58, MGL 97, MGL 105
Internode length (>16.5 cm)	RD 2902, MGL 17, MGL 40, MGL 45, MGL 57, MGL 61, MGL 97, MGL 117
Inclination angle (=90^0)	MBGSN 145, MBGSN 147, MGL 46, MGL 62, MGL 64, MGL 70, MGL 71, MGL 73, MGL 75, MGL 81, MGL 105, MGL 106, MGL 117, MGL 127, MGL 128, MGL 133, MGL 159, MGL 174, MGL 186
Tillers per meter (>140)	MGL 10, MGL 43, MGL 47, MGL 69
Spike length (>10 cm)	MGL 12, MGL 15, MGL 48
1000 grain weight (>45.0 g)	MBGSN 145, MGL 17, MGL 46, MGL 81, DWRUB 52
Grain yield (>800 g/plot)	MBGSN 145, MGL 15, MGL 58, MGL 105, MGL 117, MGL 174, RD 2552, DWRB 101, BH 946

High heterotic cross combinations are important in self pollinated species that may yield transgressive segregant in advanced generations. To achieve high F_1 heterosis, the parental divergence is very important. It has been shown that very high or very low parental divergence did not result in heterosis (Srivastava and Arunachalam, 1977). The frequency of heterotic crosses and the magnitude of heterosis were found to be higher in crosses between the parents in intermediate divergence classes than extreme ones (Arunachalam *et al.*, 1984). For improvement of a particular component trait, the promising donors, thus identified in this study and are represented in different clusters could be used in crossing programmes to obtain high heterotic response and thus better segregants in subsequent generations for higher grain yield in barley. However, further studies on specific combining ability of these genotypes needs to be investigated to obtain desirable outcome.

Acknowledgment

The authors expresses sincere gratitude to Head, Wheat and Barley Section (GPB), CCS HAU, Hisar-125004 for providing research facilities and ICAR-IIWBR, Karnal for sharing the experimental material.

Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author Contribution

Conceptualization: YK, KDS; Initial Draft: YK, KDS; Critical review and finalization: JS and SS

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