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Identification of Genetically Diverse Lines for Yield, Yield Components and Quality Characters in Promising Barley (*Hordeum vulgare* L.) Genotypes

Sunil[1], K. D. Sehrawat[1] and Mujahid Khan[2*]

[1]Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India.
[2]Department of Mathematics, Statistics and Physics, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author Sunil designed and performed the study, wrote the protocol and the first draft of the manuscript. Author KDS managed the literature searches and guided authors Sunil and MK during whole study period. Author MK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Barley germplasm comprising of 170 accessions (101 two rowed and 69 six rowed) along with 3 standard check varieties (1 two rowed and 2 six rowed) were evaluated to determine genetic diverse lines for grain yield and yield components in an augmented block design consisting of 10 complete blocks during *rabi* season of 2014-15 at CCS Haryana Agricultural University, Hisar, Haryana. The Non-hierarchical Euclidean cluster analysis grouped the genotypes including checks into eleven and nine distinct clusters for two and six rowed barley genotypes respectively, indicating existence of high degree of genetic diversity in the genotypes evaluated. Representative genotypes from a cluster could be chosen for hybridization program. The clusters formed in divergence analysis have genotypes of heterogeneous origin thereby indicating no parallelism

**Corresponding author: E-mail: mkhanstat@gmail.com;*

between genetic and geographic diversity. The highest intra-cluster distances were recorded for cluster XI (14.37) and cluster I (12.06) in two and six rowed barley respectively, which indicates the existence of maximum variability within the clusters. The crossing between superior genotypes of above diverse cluster pairs can provide desirable transgressive segregates for developing high yielding varieties of barley.

Keywords: Two rowed barley; six rowed barley; cluster; distance; diversity.

1. INTRODUCTION

Genetic divergence is a process of one species diverging into more than one species over time, *i.e.* passing small random changes from one generation to next generation. The breeders are interested to evaluate genetic divergence based on morphological traits because they are inexpensive, rapid and simple to score. The study of these traits needs neither sophisticated method nor complicated equipment and also these traits can be inherited without either specific biochemical or molecular techniques. Until now scientific classification of plant was based on morphological traits (Kumar [1]).

Mahalanobis [2] outlined a statistical procedure 'D^2 statistic' to measure the genetic divergence in a given population. This concept is based on the technique of utilizing measurement in respect to an aggregate of characters. Multivariate analysis has been considered as an important tool in quantifying the degree of genetic divergence in different crops (Rao [3]). Varieties from different localities are generally included in the hybridization programmes assuming genetic diversity and greater chances of recovering promising segregates. However, Murthy and Anand [4] noted that there is no parallelism between geographical and genetic diversity.

Genetic divergence among the genotypes plays an important role in selection of parents having wider variability for different traits (Nayak et al. [5]). The ultimate goal of any plant breeding programme is to develop improved genotypes which are better than the existing ones in producing the economic yield. This requires genetic amelioration through maximum utilization of allelic resources to develop ideal genotype.

Barley is one of the agronomically most important cereals and has the ability to grow and produce in marginal environments, low temperature and salinity. In order of importance, barley is used for animal feed, brewing malts and human consumption (Hays et al. [6]). By products from the brewing process and malt sprouts are also used in livestock feed. Barley provides a good source of energy since barley kernel constitutes 80% carbohydrates. The delicious and energetic ready to serve foods like horlics, maltova and ovaltine include barley as one of the major component. Barley has been considered a high energy food since the Roman times, when the gladiators in ancient Rom were called "hordeari" (from *Hordeum*) because they were fed on barley diet before going to the Circus. Recently it has been found that the non-starch polysaccharides in barley, known as β-glucan can lower the blood serum cholesterol level in human.

Barley has characteristically two rowed and six rowed inflorescence with the preponderance of latter as per record of released varieties. Thus, it was important to identify promising genotypes for two rowed and six rowed barley which are genetically diverse and then to evaluate these promising genotypes for important qualitative characters.

2. MATERIALS AND METHODS

The barley germplasm lines used in the study consisted of one hundred seventy accessions (101 two rowed and 69 six rowed) and three check varieties (BH393 six rowed, BH946 six rowed and BH885 two rowed). The experimental work was conducted under irrigated-normal soil conditions during 2014-15 *rabi* season at Wheat and Barley section research farm, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The augmented randomized block design introduced by Federer [7] was used for the experiment. An augmented experimental design is usually useful for testing a large number of genotypes in early generations when valid statistical analyses are needed particularly when seed supplies are too limited to permit replication. The basic concept of augmented design construction is to establish a standard replicated design using checks for which sufficient seeds are available.

Three checks were repeated in all the ten blocks along with 17 test entries in a 2.5 m long single

row plot. Row to row distance of 30 cm and plant to plant distance of 10 cm was maintained. The recommended package of practices was followed to raise a good crop. Observations were recorded from five competitive plants of each accession. The data were recorded on the characters days to flowering (75%), days to maturity (75%), plant height (cm), peduncle extrusion length (cm), number of tillers/plant, number of grains/spike, 1000 seed weight (g), grain yield/plant (g), biological yield/plant (g) and harvest index (%). Average of the data from the sampled plants of each plot in respect of different characters was used for various statistical analyses.

Genetic divergence among 173 genotypes including checks planted in augmented design was studied through Non-hierarchical Euclidean cluster analysis (Beale [8], Spark [9]). Mahalanobis [2] D^2 analysis was used for assessing the genetic divergence among the test genotypes involving quantitative characters. The generalized distance between any two populations was given by the formula:

$$D^2 = \sum\sum \lambda_{ij} \sigma_{ai} \sigma_{aj}$$

Where,

D^2 = Square of generalized distance,

λ_{ij} = Reciprocal of the common dispersal matrix,

σ_{ai} = $(\mu_{i1}-\mu_{i2})$,

σ_{aj} = $(\mu_{j1}-\mu_{j2})$ and

μ = General mean

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized characters (X's) to standardized uncorrelated variables (Y's) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (s) values of any two uncorrelated genotypes (Rao [10]).

All n (n-1)/2 D^2 values were clustered using ward minimum method described by Rao [10]. The intra-cluster and inter-cluster distances were calculated by the formula given by Singh and Choudhary [11], respectively as:

$$\text{Square of the intra cluster distance} = \frac{\sum D_i^2}{n}$$

$$\text{Square of the inter cluster distance} = \frac{\sum D_i^2}{n_i n_j}$$

Where,

$\sum D_i^2$ was the sum of distances between all possible combinations of the genotypes included in a cluster.

n = Number of all possible combinations

n_i = Number of genotypes in cluster i

n_j =Number of genotypes in cluster j

The promising genotypes which were selected on the basis of diversity analyses then are evaluated at National Dairy Research Institute (NDRI), Karnal against four qualitative traits viz. hectoliter weight, grain plumpness (%), protein content (%) and malt yield (%).

3. RESULTS AND DISCUSSION

The genetic divergence existed in 173 (170 germplasm lines and 3 checks) barley genotypes, was studied by employing Non-hierarchical Euclidean cluster analysis for ten quantitative characters. One hundred two genotypes of two rowed barley (101 germplasm lines and 1 check) were grouped into eleven different non-overlapping clusters (Table 1) and 71 genotypes of six rowed barley (69 germplasm lines and 2 checks) were grouped into nine different non-overlapping clusters (Table 2). The discrimination of lines into so many discrete clusters suggested presence of high degree of genetic diversity among the genotypes evaluated, which indicated that this material may serve as good source for selecting the diverse parents for hybridization programme aimed at isolating desirable segregates for developing high yielding varieties of barley. Clustering pattern of genotypes showed lack of any relationship between geographic origin and genetic diversity. This finding is in conformity with the previous reports advocating lack of parallelism between genetic and geographic diversity in barley by Negassa [12], Demissie and Bjornstad [13], Alam et al. [14], Ahmad et al. [15] and Muhe and Assefa [16].

The estimates of intra and inter-cluster distance for eleven clusters in two rowed barley genotypes are presented in Table 3. The highest intra-cluster value was found for cluster XI (14.37) followed by cluster VIII (13.60) and cluster X (13.50). The maximum inter-cluster distances were recorded among cluster V and cluster X (56.46) followed by cluster VI and X (52.83). The estimates of intra and inter-cluster distance for nine clusters in six rowed barley genotypes are presented in Table 4. The highest intra-cluster value was found for cluster I (12.06) followed by cluster V (10.58) and cluster IX (9.95). The maximum inter-cluster distances were recorded among cluster III and cluster VI (63.65) followed by cluster VI and VII (62.11). An examination of the estimates of within and between cluster, genetic diversity presented as intra and inter-cluster values revealed that genotypes of the same cluster had little divergence from each other with respect to aggregate effect of the characters studied. Therefore, the chances of obtaining good recombinants in segregating generations by crossing the members of the same cluster will be very low. Similar results were also reported by Abebe et al. [17], Muhe and Assefa [16] and Shinde et al. [18].

The cluster means for ten characters are presented in Table 5 and Table 6 for two and six rowed barley genotypes, respectively. The inter-cluster group means for ten characters revealed considerable differences between the clusters in respect of cluster means. So crossing between the genotypes belonging to cluster pairs separated by larger inter-cluster distances and having high cluster means for one or another character to be improved is likely to be more fruitful. The intra-cluster group means for ten characters revealed considerable differences between the genotypes within a cluster in respect of cluster means. The genotypes belonging to these clusters figured important to be included in crossing programme to further expand genetic variability among populations, to affect selection of elite lines for hybridization programme. The findings were supported by previous studies of Ahmad et al. [15], Akgun et al. [19], Kumar et al. [20] and Al-Yassin et al. [21].

Table 1. Clustering pattern of 102 (101 test genotypes + 1 check) two rowed barley genotypes for ten characters

Cluster	Number of genotypes	Genotypes
I	13	20thIBON3, DWR30, ICARADA54, BCU554, BH963, VJM522, BH964, EIBGN2013-14-54, DWRUB76, BK306, VJM516, DWR80, IstGBON2012-13-167
II	15	BCU4968, EIBGN2013-14-57, IstGBON2012-13-12, PL880, DWR49, BK1107, IstGBON2012-13-235, BK1127, BH12-29, IstGBON2012-13-150, RD2784, KB1367, UPB1042, BCU2237, KB1363
III	10	SLOOP SAW I 3167, SLOOP VIC VB9953, BCU1224, SK9, 1stGBON2012-13-9, DWR28, IstGBON2012-13-87, EIBGN2013-14-27, BH990, Alfa93
IV	15	EIBGN2013-14-55, EIBGN2013-14-69, RD2892, RD2897, RD2894, RD2898, EIBGN2013-14-25, DWRB123, DWRB126, DWRB125, BH988, DWRB128, DWRB124, DWRUB73, RD2896
V	2	SK17, SK18
VI	9	BCU2241, IstGBON2012-13-163, BK303, EIBGN2013-14-7, DWR62, DWRUB55, DWR96, DWRB92, BH987
VII	12	EIBGN2013-14-8, DWRB122, DWRB91, KB154, RD2891, RD2895, BCU4966, BH989, PL874, K1349, RD2893, DWRB127
VIII	8	DWRUB52, EIBGN2013-14-46, BH976, DWR97, EIBGN2013-14-70, BH959, EIBGN2013-14-72, BH992
IX	6	BCU5915, IstGBON2012-13-130, IstGBON2012-13-149, RD2668, 2ND INFBON131, BH10-30
X	3	BCU5783, BCU6304, BH965
XI	9	DWRB107, BHS414, BHS416, BHS419, HBL711, HBL712, RD2870, BH885, BK316

Table 2. Clustering pattern of 71 (69 test genotypes + 2 checks) six rowed barley genotypes for ten characters

Cluster	Number of genotypes	Genotypes
I	13	BCU2030, RD2832, BCU277, IstGBON2012-13-208, VLB132, BCU4980, IstGBON2012-13-265, IstGBON2012-13-261, Carafe, IstGBON2012-13-231, UPB1031, IstGBON2012-13-258, Azad
II	9	IstGBON2012-13-33, IstGBON2012-13-190, IstGBON2012-13-230, NDB1758, IstGBON2012-13-34, IstGBON2012-13-65, BHS400, IstGBON2012-13-101, IstGBON2012-13-119
III	11	K551, Manjula, BH902, BCU93, BHS415, K14, Jagriti, Lakhan, EIBGN2013-14-73, IstGBON2012-13-2, EIBGN2013-14-10
IV	6	Bilara2, K141, BH972, HUB 236, IstGBON2012-13-259, IstGBON 2012-13-262
V	11	Amber, BCU4982, BCU5070, BCU6057, BCU5173, EIBGN2013-14-11, BCU5474, EIBGN2013-14-74, IstGBON2012-13-290, EIBGN2013-14-75, IstGBON2012-13-175
VI	1	HBL276
VII	2	Kasota, BH393
VIII	10	Karan741, BH962, RD2876, PL881, RD2833, NDB1580, RD2875, RD2877, EIBGN2013-14-71, BH982
IX	8	IstGBON2012-13-29, IstGBON2012-13-84, IstGBON2012-13-195, IstGBON2012-13-228, HBL713, IstGBON2012-13-94, IstGBON2012-13-238, BH946

Table 3. Average intra and inter-cluster distances for eleven clusters in two rowed barley genotypes

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**8.54**	13.38	14.74	14.10	40.48	12.27	15.85	19.36	19.48	42.63	21.73
II		**9.22**	17.36	17.36	43.15	11.35	15.18	22.30	27.94	51.11	24.66
III			**8.81**	17.42	36.47	20.46	19.89	22.60	23.09	47.08	23.27
IV				**9.78**	40.68	16.89	15.06	18.20	22.32	39.11	21.66
V					**5.16**	44.23	40.69	48.82	48.09	56.46	36.17
VI						**6.06**	10.66	27.08	27.83	52.83	22.28
VII							**7.20**	24.14	32.16	52.22	23.65
VIII								**13.60**	16.71	24.72	21.19
IX									**6.03**	17.31	19.27
X										**13.50**	26.54
XI											**14.37**

Bold figures indicate the intra-cluster distance

Table 4. Average intra and inter-cluster distances for nine clusters in six rowed barley genotypes

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX
I	**12.06**	13.55	15.05	28.62	31.33	58.41	25.38	19.45	21.38
II		**8.70**	16.32	26.51	32.00	52.61	28.77	19.96	16.78
III			**9.27**	19.08	26.87	63.65	25.48	15.85	19.08
IV				**7.89**	13.24	41.49	41.79	14.59	17.57
V					**10.58**	25.39	40.62	16.18	17.81
VI						**0.00**	62.11	36.08	34.43
VII							**9.73**	16.38	30.29
VIII								**6.62**	14.84
IX									**9.95**

Bold figures indicate the intra-cluster distance

Table 5. Cluster means for ten characters in two rowed barley genotypes

Cluster	Days to 75% flowering	Days to 75% maturity	Plant height (cm)	Peduncle extrusion length (cm)	No. of tillers/ plant	No. of grains/ spike	1000 seed weight (g)	Grain yield/ plant (g)	Biological yield/ plant (g)	Harvest index (%)
I	77.35	133.33^{H}	104.49	6.63	10.41	23.18	48.06	7.72	20.17	38.39
II	79.56	128.73	105.03	7.09	10.96	24.11	46.70	8.04	22.10^{H}	36.44
III	95.00	132.67	104.61	5.19^{L}	10.37	22.43	45.98	7.56	20.99	36.16^{L}
IV	84.96	130.40	106.12	7.29	8.71	23.52	52.07	7.52	19.88	37.97
V	98.20^{H}	130.84	75.24^{L}	6.12	9.87	23.76	50.84	7.72	20.19	38.46
VI	77.76	128.74	106.28^{H}	6.03	11.27^{H}	24.35^{H}	51.96	8.17^{H}	20.41	40.09^{H}
VII	89.48	129.03	106.24	8.30	11.01	23.79	55.97^{H}	7.95	20.08	39.70
VIII	81.37	129.46	104.63	9.16^{H}	9.53	21.60	44.88	6.70	18.52	36.35
IX	75.89	130.50	102.54	5.29	9.76	21.81	44.10	5.87	15.88	36.97
X	75.87^{L}	126.33^{L}	95.41	7.67	8.53^{L}	19.70^{L}	42.54^{L}	5.49^{L}	14.32^{L}	38.06
XI	86.34	127.05	99.35	6.38	9.84	21.93	43.13	7.27	17.81	40.86

^{L} Lowest value, ^{H} Highest value

Table 6. Cluster means for ten characters in six rowed barley genotypes

Cluster	Days to 75% flowering	Days to 75% maturity	Plant height (cm)	Peduncle extrusion length (cm)	No. of tillers/ plant	No. of grains/ spike	1000 seed weight (g)	Grain yield/ plant (g)	Biological yield/ plant (g)	Harvest index (%)
I	79.82	131.39	103.37	6.03	7.74	35.01	47.02	8.07^{H}	22.37^{H}	36.20
II	97.94	130.96	102.64	6.58	7.90^{H}	34.44	48.52^{H}	8.00	22.12	36.09
III	80.62	130.33	108.15	9.37^{H}	7.02	33.73	45.42	7.86	20.79	37.96
IV	88.76	132.83^{H}	108.92^{H}	8.63	4.76	30.74	45.59	5.46	13.94	39.13
V	84.23	130.88	102.75	6.30	4.20	29.42	42.96	5.04	13.85	36.39
VI	100.20^{H}	131.33	75.91^{L}	3.54^{L}	3.87^{L}	26.63^{L}	41.71	4.19^{L}	12.89^{L}	32.65^{L}
VII	75.82^{L}	123.12^{L}	87.22	6.53	7.22	36.57^{H}	45.48	8.04	19.65	40.92^{H}
VIII	82.93	129.17	94.63	7.08	5.80	32.74	45.31	6.55	16.25	40.26
IX	99.18	130.00	103.62	6.16	5.93	32.36	41.55^{L}	6.88	18.46	37.40

^{L} Lowest value, ^{H} Highest value

Table 7. Contribution of different characters towards divergence in two and six rowed barley genotypes

Sr. no.	Source	Two rowed barley		Six rowed barley	
		Times ranked 1st	Contribution towards divergence (%)	Times ranked 1st	Contribution towards divergence (%)
1	Days to 75% Flowering	1917	37.21	987	39.72
2	Days to 75% Maturity	296	5.75	66	2.66
3	Plant Height (cm)	711	13.80	791	31.83
4	Peduncle Extrusion Length (cm)	57	1.11	7	0.28
5	No. of Tillers/ Plant	8	0.16	0.01	0.00
6	No. of Grains/ Spike	16	0.31	39	1.57
7	1000 Seed Weight (g)	1272	24.69	118	4.75
8	Grain Yield/ Plant (g)	575	11.17	243	9.78
9	Biological Yield/ Plant (g)	98	1.90	181	7.28
10	Harvest Index (%)	201	3.90	53	2.13

Table 8. Most promising genotypes identified from different clusters

Cluster	2 rowed barley genotypes	6 rowed barley genotypes	Characters
I	20thIBON3, BH963, VJM522, BH964, EIBGN2013-14-54, IstGBON2012-13-167	BCU2030, RD2832, BCU4980, IstGBON2012-13-265, IstGBON2012-13-261, IstGBON2012-13-231, UPB1031, Azad	Grain yield, number of grains/spike, tillers/plant, harvest index
II	EIBGN2013-14-57, PL880, IstGBON2012-13-235, BCU2237	IstGBON2012-13-33, IstGBON2012-13-190, IstGBON2012-13-230, Ist201213-34, BHS400	Grain yield, number of grains/spike, tillers/plant, harvest index
III	IstGBON2012-13-9, DWR28, IstGBON2012-13-87, BH990, ALFA93	K551, IstGBON2012-13-2, BHS415, EIBGN2013-14-10, Manjula	Grain yield, number of grains/spike, tillers/plant, harvest index
IV	RD2897, RD2898, RD2896, EIBGN2013-14-25, DWRB123, DWRB126, DWRB128, DWRB124	-	Grain yield, number of grains/spike, tillers/plant, harvest index
V	SK18	-	Grain yield, number of grains/spike, tillers/plant, harvest index
VI	BCU2241, IstGBON2012-13-163, BK303, EIBGN2013-14-7, BH987	-	Grain yield, number of grains/spike, tillers/plant, harvest index
VII	DWRB122, BCU4966, BH989, PL874, K1349, DWRB127, RD2893	Kasota	Grain yield, number of grains/spike, tillers/plant, harvest index
VIII	EIBGN2013-14-46	PL881, NDB1580, RD2877, BH982	Grain yield, number of grains/spike, tillers/plant, harvest index
IX	-	IstGBON2012-13-29, HBL713, IstGBON2012-13-84, IstGBON2012-13-195, IstGBON2012-13-228	Grain yield, number of grains/spike, tillers/plant, harvest index
XI	DWRB107, RD2870, BH885, BK316	-	Grain yield, number of grains/spike, tillers/plant, harvest index

Days to 75% flowering, 1000 seed weight, plant height and grain yield/plant were the main contributors towards divergence in two rowed barley as well in six rowed barley (Table 7). It may be concluded that the greater divergence in the genotypes due to these characters in the respective clusters would offer a good scope for the improvement of barley through selection. Ram and Singh [22], Nessa et al. [23] and Alam et al. [14] also reported similar results in barley crop.

The promising genotypes selected from different clusters are listed in Table 8 along with the list of characters for which they attributed superior performance. The promising genotypes were selected from clusters which have higher mean than the general mean as well as cluster mean, for the grain yield and its associating characters.

All the promising genotypes were evaluated for hectoliter weight, grain plumpness, protein content and malt yield in both the two rowed genotypes and six rowed genotypes. Among the 69 promising genotypes, hectoliter weight (kg/hl), grain plumpness (%), protein content (%) and malt yield (%) were measured highest in BH963 (63.5), BH989 (98.53), BCU4966 (13.7) and DWRB126 (92.12) genotypes, respectively.

The genotypes PL880, BCU2237, BHS5400, Manjula, K551, Kasota, BCU4980, BHS415, UPB1031, BCU2030, Azad, and EIBGN2013-14-10 were isolated to be the most significant and useful genotypes keeping in view the yield and its associating traits. They were good in their quality attributes also. These genotypes should be utilized in breeding programme for the improvement of barley.

4. CONCLUSION

The Non-hierarchical Euclidean cluster analysis grouped the genotypes including checks into eleven and nine distinct clusters for two and six rowed barley genotypes respectively, indicating existence of high degree of genetic diversity in the genotypes evaluated. The distribution pattern of genotypes in different clusters was random and there was little association of genetic divergence with place of origin of genotypes. The highest intra-cluster distance was recorded for cluster XI in two rowed barley and for cluster I in six rowed barley, which indicates the existence of maximum variability within the clusters. Inter-cluster distance was maximum between clusters V and X in two rowed barley while between clusters III and VI in six rowed barley, which indicates that the genotypes included in these clusters are genetically diverse. The cluster means for all the ten characters studied in barley genotypes revealed considerable differences among all the clusters. The character days to 75% flowering contributed maximum towards genetic divergence. The promising genotypes were selected from each cluster and these were found important to be included in crossing programme to further exploit genetic variability among populations, to affect selection of elite genotypes for hybridization programme and/or elite populations for transgressive segregates with high genetic potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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