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Research Article

Hierarchical cluster analysis in barley genotypes to delineate genetic diversity

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Abstract
The multivariate technique of hierarchical cluster analysis in 87 barley genotypes indicated substantial genetic diversity in the experimental material. The experiment was conducted at Barley Research Area of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Rabi* 2016-17. The estimates of coefficient of variation (CV) were observed highest for the number of grains per spike whereas, days to heading and maturity exhibited the lowest coefficient of variation. UPGMA method with city block distance was used to classify the genotypes and eight clusters were formed having one to thirty one genotypes. Maximum intra-cluster distance was observed for cluster V (38.85) followed by cluster VI (37.20) whereas; it was recorded minimum for cluster VII. The average inter-cluster distance was found to be highest between the clusters II and V (132.11) followed by clusters I and II (126.17) while the lowest inter-cluster distance was observed between clusters V and VI (53.37). The improvement in six rowed barley could be achieved through the use of genotypes assigned in clusters I and IV, whereas the genotypes which contained in cluster II and VII might be considered as potential parents for two rowed barley to obtain high heterotic response and accordingly better segregants for grain yield. The hierarchical cluster analysis adopted in this investigation proved to be very effective and helpful in isolating the most diverse promising genotypes for future study.

Key words
Barley, Cluster analysis, Genetic diversity

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the major annual cereal grains, currently ranking fourth behind rice, wheat and maize in the world production. This crop has potential to grow under drought and saline conditions. It requires less input such as fertilizers, irrigation, and insecticides. Barley grain is used as feed, food, and malting purposes, while straw provides an important source of roughage for animals particularly in the dry areas. In the modern time, it is also preferred as medicinal food in urinary as well as cardiac problems. The changing climatic scenario in country for temperature, rainfall and crop duration has made it a potential crop for near future (Raikwar, 2015). In India, the area under barley during the crop season 2018-19 was 0.66 million hectare with the production and average productivity of 1.73 million tonnes and 26.17 q/ha, respectively. Haryana state achieved a production level of 57,990 tonnes on 18,100 hectares. The average crop productivity in barley is highest in Punjab (3800 kg/ha) followed by Haryana (3204 kg/ha), Rajasthan (2950 kg/ha) and Uttar Pradesh (2801 kg/ha) [ICAR-IIWBR, 2019].

Genetic diversity is defined as the amount of genetic variability which is reflected by differences of DNA sequence, biochemical characteristics, physiological properties or morphological characters among individuals of a variety or a population (Filiz, 2012). Study on genetic diversity is the process that analyzes the variation among

genotypes by a specific method or combination of methods. The use of cluster analysis algorithms is an important strategy for classifying germplasm, ordering variability for a large number of accessions, or analyzing genetic relationships among materials. This statistical analysis has several advantages [Peeters and Martinelli, 1989]. First, it allows mixing of both qualitative and quantitative data and therefore all the available information on the sample can be utilized, it can serve as a tool of selection and data reduction via similarity coefficient, similar genotypes may consider one genotype in the second test of performance provided that they have genetic diversity among them to avoid inbreeding effect. Also, it provides useful information about genetic diversity in crops. Cluster analysis had been used in widely different fields (Ibrahim *et al.,* 2011).

Efficient utilization of genetic potential hidden in elite genotypes requires detailed knowledge about the material under study. Such knowledge can provide major reservoir of genetic diversity useful for genetic improvement of a crop (Bhatt, 1970). Identifying, quantifying and utilizing genetic diversity is essential to meet future demands for crop cultivars (Strauss *et al*., 1988). Obviously, conservation and evaluation of breeding material is of paramount concern for its effective utilization.

It is widely accepted that evaluation and cataloguing of genetic resources is an essential prerequisite for a successful breeding programme, which facilitates utilization of diverse germplasm (Tewari *et al*., 2015). Genotypes that have not been systematically characterized can contain duplicate or too many unique or rare types. Calculation of genetic distances can identify divergent genotypes that could harbour valuable genetic variations. Hierarchical cluster analysis offers solution to this problem by defining degree of relatedness in the samples and the best basis to define commonness, thereby, eliminating redundancy and characterizing degree of diversity (Peeters and Martinelli, 1989). Hence, the present study was undertaken to understand the genetic diversity among barley genotypes for different traits. This information will be useful to strengthen breeding efforts for developing improved barley varieties by utilizing the diverse sources.

MATERIALS AND METHODS

A set of 87 barley genotypes were evaluated in randomized block design with three replications at Barley Research Area of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Rabi* 2016-17. Each genotype was grown in six rows with a plot size of 5 x 1.38 m^2. Recommended package of practices were applied to raise the crop. Observations were recorded on 10 quantitative traits, *viz*., days to heading, days to maturity, plant height (cm), spike length (cm), the number of tillers per meter, the number of grains per spike, 1000 grain weight (g), harvest index (%), biological yield (kg/plot) and grain yield (kg/plot). Five randomly selected competitive plants in each replication were recorded for all the traits under study except of days to heading, days to maturity, biological yield and grain yield which were recorded on plot basis. Further, the values of harvest index were calculated as per the formula given by Donald and Humblin (1976).

The estimates of variability parameters were calculated following standard statistical procedures. 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, City Block distance (also known as Manhattan) measure was used to find out the relative distances between and within the different clusters. For two cases, it is the sum of the absolute differences of the values for all the variables. Multivariate cluster analysis may be a very useful method in interpreting the results of agricultural experiments (Klikocka and Tatarczak, 2015).

Table 1. Estimates of genetic variability for different characters

Characters	Mean ±SE (m)	Range	Standard deviation (SD)	Coefficient of Variation (CV)
Days to heading	81.79±0.31	76.0-88.0	2.89	3.53
Days to maturity	122.65±0.26	118.0-128.0	2.46	2.01
Plant height (cm)	101.8±1.10	73.0-125.0	10.23	10.05
Spike length (cm)	6.66±0.07	5.3-8.1	0.69	10.29
No. of tillers per meter	112.41±2.24	76.0-150.0	20.87	18.57
No. of grains per spike	43.02±2.11	21.0-76.0	19.68	45.74
1000-grain weight (g)	46.24±0.74	31.9-62.1	6.92	14.97
Biological yield (kg/plot)	8.42±0.14	5.57-11.97	1.32	15.64
Harvest index (%)	28.39±0.57	20.22-42.75	5.28	18.58
Grain yield (kg/plot)	2.36±0.05	1.36-3.60	0.47	20.24

RESULTS AND DISCUSSION

The analysis of variances revealed significant genotypic differences for all the characters under study indicated substantial genetic variability in the experimental material. The estimates of genetic variability are provided in **Table 1**. In general, the results under investigation reflected wide range for all the traits. Estimates of coefficient of variation (CV) were observed highest for the number of grains per spike followed by grain yield whereas, days to heading and maturity exhibited the lowest coefficient of variation. Remaining traits indicated moderate coefficient of variation, recommended that the selection based on these characters would facilitate successful isolation of desirable plant types. Similar findings for one or more characters have also been delineated by Kumar *et al.* (2013), Singh *et al.* (2015) and Yadav *et al.* (2015) in barley.

The hierarchical cluster analysis grouped the genotypes into eight clusters as depicted in **Table 2**. The membership profile recognized cluster III as largest one with thirty one genotypes followed by cluster V (20), VI (13) and cluster II (11), while the cluster VII was the smallest with one genotype only. The clustering pattern showed that two rowed genotypes got distributed in four clusters *viz*., cluster II, III, VII and VIII however, rest of the clusters composed of six rowed genotypes. Eticha *et al.* (2010) also used Hierarchical cluster analysis to characterize and classify diverse hull-less barley genotypes based on their overall similarity in agronomic and qualitative data and also identified the genotypes that best combines both agronomic and quality characters for the future use in hull-less barley breeding. Singh *et al.* (2013) also studied the genetic divergence among 108 germplasm collections based on quantitative characters in barley.

Table 2. Cluster membership profile of different genotypes

Clusters	Genotypes	No. of genotypes
I	BH 10-11 (1), BH 10-03 (34), BH 393 (41), BH 16-37 (78), BH 16-40 (81)	5
II	BH 10-31 (2), BH 14-06 (9), BH 14-07 (10), BH 14-25 (12), BH 14-40 (21), BH 15-38 (32), DWRB 101 (35), DWRUB 52 (39), DWRB 92 (40), BH 16-25 (66), BH 16-28 (69)	11
III	BH 12-29 (3), BH 13-20 (5), BH 13-26 (7), BH 15-17 (16), BH 14-17 (20), BH 14-43 (22), BH 15-11 (26), BH 15-12 (27), BH 15-24 (29), BH 885 (36), BH 16-01 (42), BH 16-02 (43), BH 16-03 (44), BH 16-04 (45), BH 16-05 (46), BH 16-08 (49), BH 16-09 (50), BH 16-12 (53), BH 16-14 (55), BH 16-15 (56), BH 16-16 (57), BH 16-19 (60), BH 16-21 (62), BH 16-22 (63), BH 16-23 (64), BH 16-24 (65), BH 16-26 (67), BH 16-27 (68), BH 16-31 (72), BH 16-32 (73), BH 16-46 (87)	31
IV	BH 12-46 (4), BH 7-35 (19)	2
V	BH 13-22 (6), BH 15-07 (15), BH 15-30 (17), BH 7-34 (18), BH 15-06 (25), BH 15-37 (31), BH 946 (38), BH 16-06 (47), BH 16-07 (48), BH 16-11 (52), BH 16-13 (54), BH 16-17 (58), BH 16-20 (61), BH 16-33 (74), BH 16-38 (79), BH 16-41 (82), BH 16-42 (83), BH 16-43 (84), BH 16-44 (85), BH 16-45 (86)	20
VI	BH 14-01 (8), BH 14-13 (11), BH 14-42 (13), BH 15-02 (14), BH 14-44 (23), 15-16 (28), BH 15-25 (30), BH 15-39 (33), BH 902 (37), BH 16-10 (51), BH 16-18 (59), BH 16-29 (70), BH 16-30 (71)	13
VII	BH 15-05 (24)	1
VIII	BH 16-34 (75), BH 16-35 (76), BH 16-36 (77) BH 16-39 (80)	4
Total		87

Values in parenthesis indicates serial number of genotypes

The association among the different genotypes is presented in the form of dendrogram (**Fig.1**) prepared using rescaled distances. The genotypes, which are lying nearer to each other in the dendrogram, are more similar to one another than those lying apart (Brown, 1991). The resemblance coefficient between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among the different clusters. Zakova and Benkova (2006) evaluated and grouped 106 accessions of spring barley into different clusters based on multivariate analysis.

The estimates of intra and inter-cluster distances were calculated using city block distance and are presented in **Table 3**. The inter-cluster distance was higher than the intra-cluster, explaining wide genetic diversity among the genotypes. The maximum intra-cluster distance was recorded for clusters V (38.85) followed by cluster VI (37.20) and cluster III (36.57), implies that the genotypes in these clusters were relatively more diverse than the other clusters. On the other hand, minimum intra-cluster distance was observed in cluster VII since it contains 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 highest inter-cluster distance was observed between clusters II and V (132.11) followed by clusters I and II (126.17) whereas it was minimum between clusters V and VI (53.37). The inter-cluster values that indicated close relationship were to be considered that hybridization

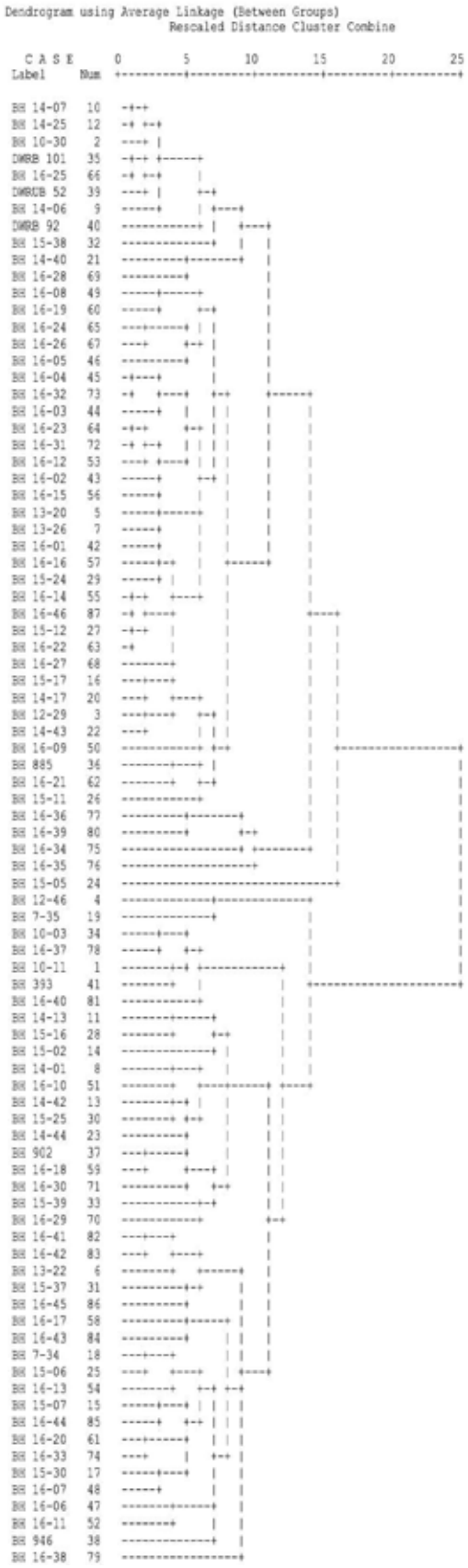

Fig. 1. Dendrogram portraying clustering pattern of different genotypes

among the genotypes of these clusters would not provide good level of segregation. 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. The hybrids developed from the selected genotypes within the limit of compatibility of these clusters may produce desirable transgressive segregants of high magnitude of heterosis. Ebrahim *et al.* (2015), Hailu *et al.* (2016), Sarkar *et al*. (2014) and Yadav *et al.* (2015) also studied and reported the existence of genetic diversity in barley.

The perusal of cluster means showed considerable differences in mean values for all characters under study (**Table 4**). Cluster I comprised of five genotypes, exhibited minimum 1000 grain weight, the number of days to maturity and plant height, and had moderately high cluster means for harvest index. The genetic distance value of these genotypes was 24.74. Cluster II consisted of eleven genotypes including national check varieties *i.e.* DWRUB 52, DWRB 92 and DWRB 101, characterized by moderately high 1000 grain weight and biological yield with highest number of tillers per meter. The genotypes of this cluster showed genetic distance of 31.28. Cluster III being largest one, had 31 genotypes having characteristic features of longest spike with highest 1000 grain weight. The genetic distance value of these genotypes was 36.57.

Cluster IV, contained two genotypes, recorded for moderately high number of tillers per meter and harvest index with highest grain yield. The genetic distance value was 19.40 for these genotypes. Twenty genotypes constituted cluster V and characterized by highest number of grains per spike with minimum number of tillers per meter, among the clusters having six row types. Maximum genetic distance (38.85) was observed between genotypes of this cluster. Late maturing genotypes are grouped into Cluster VI which consisted of 13 genotypes. The genotypes of this cluster showed a genetic distance of 37.20. The clusters VII and VIII were assigned with two rowed barley genotypes. Cluster VII consisted of early maturing genotype, exhibited maximum biological yield with the lowest harvest index. Four genotypes constituted Cluster VIII illustrated with maximum harvest index among all clusters. Genetic distance of 33.95 was explained by the genotypes of this cluster. Several genetic diversity studies have been conducted on barley based on quantitative traits in order to select genetically distant parents for hybridization (Dyulgerova *et al.*, 2016; Sarkar *et al*., 2014; Sharma *et al*., 2014).

Romesburg (1990) opened that findings of similar alternatives reduces the decision problem at two stages *i.e.* first to select the clusters that can best achieve the planning objective and second to select the best alternative within the best cluster. Most diverse and superior genotypes with desirable traits selected from

Table 3. Estimates of intra-and inter-cluster distances

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**24.74**	126.17	120.95	60.39	60.27	57.52	99.62	89.35
II		**31.28**	54.29	85.20	132.11	110.58	89.20	68.00
III			**36.57**	80.69	115.17	91.94	66.88	61.70
IV				**19.40**	72.45	54.96	105.36	85.25
V					**38.85**	53.37	77.29	115.47
VI						**37.20**	80.98	98.90
VII							**0.00**	85.10
VIII								33.95

Diagonal: Intra-cluster distances Off-diagonal: Inter-cluster distances

Table 4. Mean performance of clusters for different characters in barley

Characters	Clusters							
	I	II	III	IV	V	VI	VII	VIII
Days to heading	80	81	82	80	82	84	78	82
Days to maturity	120	122	123	122	123	124	120	122
Plant height (cm)	84	97	107	97	103	103	112	85
Spike length (cm)	6.3	6.4	6.9	6.0	6.6	6.7	6.2	6.7
No. of tillers per meter	99	145	125	125	84	103	90	116
No. of grains per spike	64	26	25	63	65	63	26	24
1000-grain wt. (g)	39.1	49.9	51.6	39.6	41.3	41.6	44.0	47.7
Grain yield (kg/plot)	2.56	2.63	2.14	2.86	2.45	2.37	2.78	2.25
Biological yield (kg/plot)	7.32	9.19	8.30	9.03	8.28	9.06	11.33	6.23
Harvest index (%)	35.28	28.94	25.83	31.99	29.80	26.41	24.50	36.67

Table 5. Diverse and superior genotypes with desirable traits selected from different clusters

Sr. No.	Characters	Desirable genotypes
1	Days to heading (Early)	Six rowed: BH 393, BH 7-35 Two rowed: BH 15-05, BH 14-06
2	Days to maturity (Early)	Six rowed: BH 393, BH 10-11 Two rowed: BH 16-15
3	Plant height (cm)	Six rowed: BH 393, BH 10-11, BH 12-46 Two rowed: BH 16-35, DWRB 92, BH 10-30
4	Spike length (cm)	Six rowed: BH 14-44, BH 13-22 Two rowed: BH 16-15, BH 16-12, BH 13-26
5	No. of tillers per meter	Six rowed: BH 12-46, BH 7-35 Two rowed: BH 14-07, DWRB 92, BH 14-25, BH 10-30
6	No. of grains per spike	Six rowed: BH 13-22, BH 16-17 Two rowed: BH 13-20, DWRUB 52
7	1000 grain wt. (g)	Six rowed: BH 15-06, BH 15-02, BH 7-34 Two rowed: DWRB 92, BH 15-17, BH 16-12
8	Grain yield (kg/plot)	Six rowed: BH 15-07, BH 15-06, BH 15-02, BH 946, BH 393, BH 7-34, BH 7-35, BH 14-44, BH 10-11, BH 12-46 Two rowed: BH 14-17, BH 14-07, BH 10-30, BH 16-12, DWRUB 52, DWRB 92, BH 14-25, BH 13-26, BH 15-05
9	Biological yield (kg/plot)	Six rowed: BH15-07, BH 7-34, BH 15-06 Two rowed: DWRUB 52, BH 15-05, BH 13-26, BH 14-17
10	Harvest index (%)	Six rowed: BH 946, BH 15-02 Two rowed: BH 16-35, BH 14-17, BH 10-30

different clusters are represented in Table 5. From this study, it can be concluded that clusters I and IV for six rowed and clusters II and VII for two rowed might be considered desirable for selecting genotypes which may be used as promising parents for hybridization. The genotypes which fall in these clusters could be used in crossing programme to obtain high heterotic response and thus better segregants in subsequent generations for higher grain yield in barley. However, for improvement of a particular character, the genotype with better mean values can be selected among all the clusters to suit for further breeding programmes.

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