Hierarchical cluster analysis in barley genotypes to delineate genetic diversity

Yogender Kumar*, Ram Niwas1, Somveer Nimbal and M.S. Dalal

Wheat and Barley Section
Department of Genetics and Plant Breeding
1Directorate of Research, CCS Haryana Agricultural University, Hisar – 125004, India.
*E-Mail: yogenderkgulia@gmail.com

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
Hierarchical cluster analysis in barley genotypes

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 m². 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, 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

---

**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     | 0.89                    | 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                         |

https://doi.org/10.37992/2020.1103.122
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.

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 15-07 (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

https://doi.org/10.37992/2020.1103.122
Yogender Kumar et al., 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 31.28. Cluster VII 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 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 quantiative 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 | 115.47| 85.10 |
| 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: BH 15-07, 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 |

https://doi.org/10.37992/2020.1103.122
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.

REFERENCES

Bhatt, G.M. 1970. Multivariate analysis approach to selection of parents for hybridization aiming at yield improvement in self-pollinated crops. Aust. J. Agric. Res., 21: 1-7. [Cross Ref]

Brown, J.S. 1991. Principal component and cluster analysis of cotton cultivars variability across U.S. cotton belt. Crop Sci., 31: 915-922. [Cross Ref]

Donald, C.M. and Humblin, J. 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Av. Agron., 28: 361-405. [Cross Ref]

Dyulgerova, B., Dimova, D. and Valcheva, D. 2016. Genetic diversity in six rowed winter barley (Hordeum sativum Jess., ssp. vulgare L.) genotypes. Bulgarian J. Agric. Sci., 22 (1): 114-118.

Ebrahim, S., Shiferaw, E. and Hailu, F. 2015. Evaluation of genetic diversity in barley (Hordeum vulgare L.) from Wollo high land areas using agro-morphological traits and hordein. African J. Biotech., 14 (22): 1886-1896.

Eticha, F., Grausgruber, H. and Berghoffer, E. 2010. Multivariate analysis of agronomic and quality traits of hull-less spring barley (Hordeum vulgare L.). J. Plant Breed. Crop Sci., 2(5): 81-95.

Filiz, E. 2012. Genetic diversity analysis of CIMMYT bread wheat (Triticum aestivum L.) lines by SRAP markers. Electronic J. Plant Breed., 3(4): 956-963.

Hailu, A., Alamerew, A., Nigussie, M. and Assefa, E. 2016. Study of genetic diversity in different genotypes of barley (Hordeum vulgare L.) based on cluster and principal component analysis. Agric. Sci. Res. J., 6 (2): 31-42.

Ibrahim, O.M., Mohamed, M. H., Tawfik, M.M. and Badr, E. A. 2011. Genetic diversity assessment of barley (Hordeum vulgare L.) genotypes using cluster analysis. Int. J. Acad. Res., 3 (2): 81-85. [Cross Ref]

ICAR-IIWBR, 2019. Director’s Report of AICRP on Wheat and Barley 2018-2019, Ed: G.P. Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India. P 72.

Klikocka, H. and Tatarczak, A. 2015. The use of cluster analysis to evaluate yield and yield components of spring barley in a two- ariable field experiment. Int. J. Agric. Stat. Sci., 11 (1): 35-42.

Kumar, Y., Lamba, R.A.S., Verma, S.R. and Niwas, R. 2013. Genetic variability for yield and its components in barley (Hordeum vulgare L.). Forage Res., 39: 67-70.

Peeters, J.P. and Martinelli, J.A. 1989. Hierarchical cluster analysis as a tool to manage variation in germplasm collections. Theor. Appl. Genet. 78: 42-48. [Cross Ref]

Raiikwar, R. S. 2015. Generation mean analysis of grain yield and its related traits in barley (Hordeum vulgare L.). Electronic J. Plant Breed., 6(1): 37-42.

Romesburg, H.C. 1990. Cluster Analysis for Researches. Krieger Publishing Co., Florida.

Sarkar, B., Sarkar, A., Sharma, R.C., Verma, R.P.S. and Sharma, I. 2014. Genetic diversity in barley (Hordeum vulgare) for traits associated with feed and forage purposes. Ind. J. Agri. Sci., 84 (5):102-107.

Sharma, A., Joshi, N., Cheema, B. S., Jindal, M. M. and Singh, S. 2014. Assessment of genetic diversity in barley (Hordeum vulgare L.). J. Res. PAU. 51(2): 105-108.

Singh, A., Seth, V. and Goswami, A. 2015. Study of genetic parameters for yield and yield contributing trait of elite genotypes of barley (Hordeum vulgare L.). Trends Biosci., 8: 898-901.

Singh, M., Vishwakarma, S. R. and Singh, A.P. 2013. Genetic divergence in barley. Progressive Res., 8 (2): 230-232.

Singh, S. K., Verma, P. N., Singh, L., Ali, T. and Prasad, K. D. 2014. Variability and divergence analysis in barley (H. vulgare L.) under irrigated condition. Trends Biosci., 7 (6): 452-456.

Strauss, M.S., Pino, T.A. and Cohen. J.I. 1988. Quantification of diversity in ex-situ plant collection. Diversity 16: 30-32.

Tewari, R., Jaiswal, J.P., Gangwar, R.P. and Singh, P.K. 2015. Genetic diversity analysis in exotic germplasm accessions of wheat (Triticum aestivum L.) by cluster analysis. Electronic J. Plant Breed., 6(4): 1111-1117.

https://doi.org/10.37992/2020.1103.122

747
Yadav, N., Verma, S.R. and Singh, S. 2015: Studies of genetic variability and trait association for grain yield and its components in two rowed and six rowed barley (Hordeum vulgare L.). Bioinfolet, 12: 521-524.

Zakova, M. and Benkova, M. 2006. Characterization of spring barley accessions based on multivariate analysis. Commun. Biometry Crop Sci., 1(2):124–134.