

Assessment of Genetic Diversity for Seed Quality Parameters in Barley

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ABSTRACT: Fifty genotypes of barley (*Hordeum vulgare*) were analyzed for genetic divergence on the basis of seed vigour parameters. The observations were recorded on seedling length (cm), seed density (g/cc), standard germination (%), seedling dry weight (mg), vigour index I & II, electrical conductivity (iS/cm/seed) and accelerated ageing at 40±1°C for 48 and 72 hours. All the genotypes were grouped into seven well defined clusters depending upon the similarity in the expression of their genetic divergence. Maximum numbers of genotypes were assigned in cluster I (18) followed by cluster IV (12) and V (6), while the cluster II and VII were the smallest one with three genotypes each. Maximum distance among the genotypes within the same cluster was manifested by cluster VII (5.42) followed by cluster II (3.71) and VI (3.63), however the minimum intra-cluster distance was exhibited by the cluster IV (2.23). Cluster VI and VII showed maximum inter-cluster distance of 7.51, followed by cluster II and VI (7.40), whereas it was minimum between cluster I and IV (3.10). Contribution of electrical conductivity towards divergence was maximum (32.24%) thereafter accelerated ageing (72 h and 48 h) which contributed 18.78% and 17.71%, respectively. The clusters II and III were composed of highly vigorous genotypes (IBYT-HI-17, 2nd GSBYT-02 (2015), BH 15-17, RD 2909, 2nd GSBYT-23 (2015), 2nd GSBSN-28 (2015), MGL-117) because of better cluster means for most of the traits studied. Therefore, genotypes of clusters II and III might be considered as promising parents for barley improvement programme.

Keywords: Barley, Seed quality parameters, Genetic divergence

Barley (*Hordeum vulgare* L.), a member of the family Poaceae, is a self-pollinated diploid species with 14 chromosomes. It is cultivated in India since ancient times and is regarded as a sacred grain. Conventionally considered as a poor man's crop, because of its low input specification and better adaptability to harsh environments, likely drought, alkalinity/ salinity and marginal lands. Barley possesses high biomass, thus the small and marginal farmers can use green barley as feed for animals. Now a days, there have been great interest in utilizing barley as a dual purpose cereal crop which can enable forage production in early season besides the grain yield later on.

In India, barley occupied about 0.69 million hectares with the production and productivity of 1.79 million tons and 2580 kg/ha, respectively. Among the major barley growing states, Rajasthan tops the list in barley production (0.81 mt) followed by Uttar Pradesh (0.45 mt) and Madhya Pradesh (0.26 mt). These three states altogether accounted for 85 per cent of the total national

barley production. Haryana state achieved a production level of 0.14 million tons on 40,000 hectares. The average crop productivity of barley is highest in Punjab (3667 kg/ha) followed by Haryana (3475 kg/ha), Rajasthan (2873 kg/ha) and Uttar Pradesh (2629 kg/ha) [1].

Around the world, number of barley varieties and genotypes are present in cultivated fields, landraces and wild species in wild habitats. To exploit the desirable traits present among them for disease resistance, yield improvement, ameliorating quality for malting, fodder barley and as food for human being, it is necessary to quantify the diversity from virtually all regions of world, in order that it can be accessible for scientists and breeders to use the appropriate genotypes for purposeful breeding programmes. There are innumerable studies in literature concerned to genetic diversity in barley based on morphological and yield attributing parameters but the information based on seed vigour parameters are very scarce.

Quality seed is a basic input in agriculture for successful crop production. It is very important to assess the quality and vigour of the seed as it stipulates which seed lot is to be sown in the field. Seed vigour has been recognized as one of the important aspects of the seed quality that provides reproducible results which are more closely related with emergence in the field. Two types of methods are commonly used for detection of seed vigour: one is by measuring the seed germination related parameters like standard germination, seedling length, seedling dry weight, vigour indices etc. and another is through detecting stress enduring ability and biochemical vigour indicators of seed. Delouche and Baskin [2] reported the importance of testing seed performance using various seed vigour tests. Vigour tests also give results of field germination under adverse environmental conditions. Euclidean cluster analysis has been recognized as a powerful tool to estimate the level of divergence between genotypes. Therefore, in the present investigation, barley genotypes were assessed for genetic diversity based on seed vigour parameters.

MATERIALS AND METHODS

The experimental material consisted of 50 diverse genotypes of barley including 17 two and 33 six rowed types. The material was evaluated in randomized block design (RBD) with three replications at Barley research area of the Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar during *rabi* 2016-17 under timely sown irrigated conditions. Each genotype was grown in three rows with a plot size of 3.0 x 0.69 m². Recommended package of practices were followed to raise the good crop.

The vigour potential of these 50 genotypes with three replications was assessed by recording eight parameters *viz.*, seedling length (cm), seed density (g/cc), standard germination (%), seedling dry weight (mg), vigour index I, vigour index II, electrical conductivity (μS/cm/seed) and accelerated ageing at 48 and 72 hours in Department of Seed Science & Technology, CCS HAU, Hisar.

For calculating standard germination, one hundred seeds of each genotypes per replication were placed in between moistened rolled towel papers and kept at 20°C in seed germinator. The final count was taken on 7th day and normal seedlings were considered for recording per cent germination [3]. Seedling length (shoot + root) was measured in centimeter by taking average of ten

randomly selected normal seedlings among them used for calculation of standard germination per replication. Those seedlings, whose length was measured, were dried in hot air oven for 24 hours at 80 ± 1°C and then their dry weight was measured. Average dry weight of each genotype was calculated and expressed in milligrams.

Seed density was measured by taking one hundred seeds per replication of each genotype and weighed on electrical balance. These seeds were dipped in water having density of 1.0 at 20°C. The volume of water replaced by the seeds was recorded and seed density was calculated by using the formula:

$$\text{Seed density} = \frac{\text{Weight of 100 seeds (g)}}{\text{Volume of water replaced by seeds (cm}^3\text{)}}$$

The seedling vigour indices were calculated [4] as follows:

$$\text{Vigour Index I} = \text{Standard germination (\%)} \times \text{average seedling length (cm)}$$

$$\text{Vigour Index II} = \text{Standard germination (\%)} \times \text{average seedling dry weight (mg)}$$

Electrical conductivity of seed leachates was measured to know the status of membrane permeability. For this, 100 healthy seeds per replication were soaked in 50 ml deionized water in 100 ml beakers. Seeds were emerged completely and beakers were covered with silver foil and these samples were kept for 24 hours at 25 °C. The electrical conductivity of the seed leachates was measured by using conductivity meter in μS/cm/seed.

For accelerated ageing test, seeds were placed in a single layer on the wire mesh trays fitted in plastic boxes. Each box contained about 40 ml of distilled water. The boxes were placed in ageing chamber after closing their lids. The seeds were aged at 40±1°C for different time intervals (48 h and 72 h) and tested for standard germination per replication of 100 seeds each. The number of normal seedlings were counted on 7th day according to the rules of International Seed Testing Association and expressed in percentage.

The performance of each genotype was embayed for statistical analysis. Analysis of variance was carried out to test the significance for each character as per the method given by Panse and Sukhatme [5]. Estimates of divergence were analyzed by using Mahalanobis [6] D² statistics. The computation of D² values and for deciding group constellations, method suggested by Rao [7] was

followed. Ward's minimum variance method [8] was used to construct the dendrogram.

RESULTS AND DISCUSSION

The analysis of variance indicated that all the genotypes studied differ significantly for all the seed vigour traits under investigation. This suggested that the genotypes exhibited a wide range of variation and also indicated the suitability of experimental material for further analysis. On the basis of D^2 values, all the genotypes were assigned into seven distinct clusters. The composition of clusters presented (Table 1 and Figure 1) revealed that cluster I had the largest number of genotypes (18) followed by cluster IV (12) and V (6), while the cluster II and VII were the smallest with three genotypes each. Cluster III and VI composed of four genotypes each. Singh *et al.* [9] also studied the genetic divergence among 108 germplasm collections of barley and grouped them into 11 clusters revealing the presence of considerable amount of genetic diversity in the material. The clusters were formed by

Table 1. Clustering pattern of different barley genotypes

Clusters	Name of genotypes	No. of genotypes
I	IBYT-HI-19 (1), IBYT-HI-20 (8), 2 nd GSBSN-60 (2015) (21), DWRB 143 (40), IBYT-HI-13 (2), IBYT-HI-16 (4), BH 15-30 (47), MGL 105 (27), RD 2904 (13), BH 13-22 (42), BH 885 (49), IBYT-HI-23 (6), JB 481 (20), DWRB 101 (26), UPB 1059 (15), MGL-58 (23), AZAD (39), BH 13-26 (43)	18
II	IBYT-HI-17 (3), 2 nd GSBYT-02 (2015) (22), BH 15-17 (46)	3
III	RD 2909 (14), 2 nd GSBYT-23 (2015) (18), 2 nd GSBSN-28 (2015) (17), MGL-117 (28)	4
IV	IBYT-HI-15 (7), BH 13-20 (41), DWR 123 (10), BH 902 (34), DWR 137 (11), MGL-62 (24), BH 959 (9), K 560 (19), INBON-15-22 (37), BH 14-42 (45), HUB 242 (16), DWRUB 52 (50)	12
V	IBON-HI-1 (2015-16) (29), IBON-HI-37 (2015-16) (32), IBON-HI-3 (2015-16) (30), INBON-15-16 (36), BH 14-25 (44), BH 946 (48)	6
VI	IBON-HI-13 (2015-16) (31), IBON-HI-67 (2015-16) (33), IBYT-HI-18 (5), MGL-64 (25)	4
VII	2 nd GSBSN-15-8 (35), 2 nd GSBSN-15-35 (38), MBGSN 145 (12)	3
Total		50

Values in parenthesis indicates serial number of genotypes

assembling all the 50 genotypes in such a way that genotypes within each cluster had higher inter-cluster distances than intra-cluster distances, which is in affirmation with the study of Mittal *et al.* [10], indicated broad genetic diversity among the genotypes of different clusters than those of same cluster.

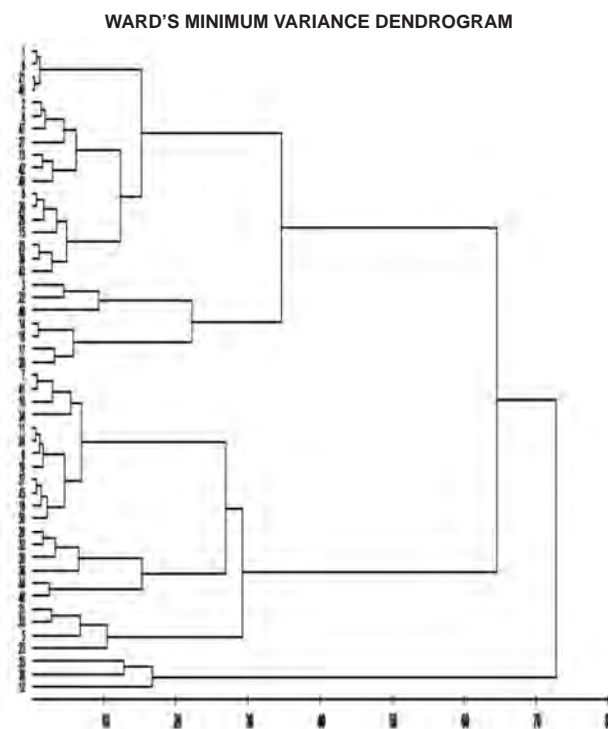


Figure 1. Dendrogram showing the clustering pattern of barley genotype

The estimates of intra- and inter-cluster distances are presented in Table 2 & Figure 2. Maximum distance among the genotypes within the same cluster was manifested by cluster VII (5.42) followed by cluster II (3.71) and VI (3.63), while the minimum intra-cluster distance was exhibited by the cluster IV (2.23). When diversity between clusters was studied it showed a range of 3.10 to 7.51. Cluster VI and VII showed maximum inter-cluster distance of 7.51, followed by that between cluster II and VI (7.40). The lowest inter-cluster distance was observed between cluster I and IV (3.10). The genotypes belong to clusters which have more distance (cluster VI and VII) are more divergent genetically. Kumar *et al.* [11] also stated that greater the distance between clusters, wider would be the genetic diversity between the genotypes. Several other studies have also been conducted to select genetically dissimilar parents for hybridization in barley [12, 15].

Table 2. Estimates of intra-and inter-cluster distances

Clusters	I	II	III	IV	V	VI	VII
I	2.689	4.707	3.583	3.099	4.073	4.679	6.558
II		3.708	4.472	4.747	5.233	7.403	6.646
III			2.561	4.199	4.852	6.317	6.860
IV				2.228	3.713	4.075	6.349
V					3.391	4.715	5.889
VI						3.627	7.508
VII							5.420

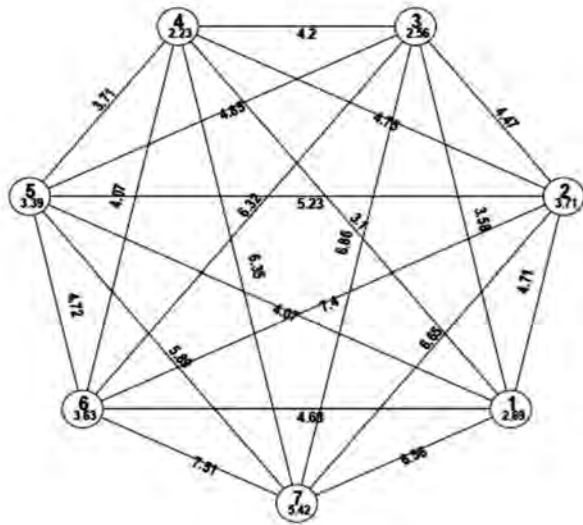


Figure 2. Mahalanobis Euclidean distances in barley genotypes

The cluster means for eight characters studied in barley genotypes revealed considerable differences among all the seven clusters (Table 3). From the current study, it is evident that cluster I, which comprised of 18 entries exhibited highest standard germination (99.43%). Cluster

II, consisted of three genotypes was characterized by highest seedling dry weight (214.83 mg) as well as vigour index II (21125.82) and also manifested high values for other traits such as seedling length (38.30 cm), seed density (1.64 g/cc), vigour index I (3766.51) and accelerated ageing (85.56) at 48 hours. The entries of cluster III performed best for seedling length (39.95 cm), vigour index I (3938.38) and accelerated ageing (87.33 & 77.67) at 48 and 72 hours, respectively. Cluster IV was represented by 12 genotypes, expressed moderately high value for trait *i.e.* accelerated ageing (74.22) at 72 hours. Cluster V, comprised of six entries recorded with highest seed density (1.75 g/cc) and also had lowest electrical conductivity (0.28). The genotypes of cluster VI also had characteristic feature of high standard germination (99.42%). Three genotypes constituted cluster VII and was characterized by moderately high seedling dry weight (183.71) and vigour index II (17751.10) along with lower values of electrical conductivity (0.29). Similar findings were also reported by various researchers in wheat [16], oat [17] and durum wheat [18] based on seed vigour parameters.

SL: Seedling length, SD: Seed density, SG: Standard germination, SDW: Seedling dry weight, VI: Vigour index I, VII: Vigour index II, EC: Electrical conductivity, AA 48: Accelerated ageing (48 h), AA 72: Accelerated ageing (72 h)

The contribution of electrical conductivity towards divergence was found maximum (32.24%) followed by accelerated ageing (72 h and 48 h) which contributed 18.78% and 17.71%, respectively. Seedling dry weight and seed density also contributed 11.76% and 11.18%, respectively towards divergence. Whereas, the remaining traits like vigour index I (3.51%) & II (2.29%), seedling

Table 3. Mean performance of different clusters for seed vigour traits

Clusters/ Characters	SL	SD	SG	SDW	VI	VII	EC	AA48	AA72
I	37.41	1.10	99.43	168.36	3715.62	16721.94	0.32	83.67	72.48
II	38.30	1.64	98.78	214.83	3766.51	21125.82	0.32	85.56	67.78
III	39.95	1.36	98.67	172.70	3938.38	17023.86	0.39	87.33	77.67
IV	35.08	1.13	98.81	173.34	3453.87	17071.88	0.33	84.39	74.22
V	35.84	1.75	98.67	161.73	3525.92	15916.38	0.28	72.22	65.56
VI	33.17	1.02	99.42	136.45	3297.25	13567.58	0.30	78.33	68.67
VII	37.60	1.30	97.44	183.71	3628.20	17751.10	0.29	40.00	32.00

SL: Seedling length, SD: Seed density, SG: Standard germination, SDW: Seedling dry weight, VI: Vigour index I, VII: Vigour index II, EC: Electrical conductivity, AA 48: Accelerated ageing (48 h), AA 72: Accelerated ageing (72 h)

length (2.53%) and standard germination (0.00%) contributed too little to divergence (Table 4). The contribution of various characters towards the expression of genetic divergence should be taken into account as a criterion for choosing parents for hybridization programmes for the improvement of such characters. Most diverse and superior genotypes with desirable traits selected from different clusters are represented in table 5.

Table 4. Contribution of different characters towards divergence

Sr. No.	Source	Times ranked 1 st	Contribution towards divergence (%)
1	Seedling length (cm)	31	2.53
2	Seed density (g/cc)	137	11.18
3	Standard germination (%)	0	0.00
4	Seedling dry weight (mg)	144	11.76
5	Vigour index I	43	3.51
6	Vigour index II	28	2.29
7	Electrical Conductivity (μ S/cm/seed)	395	32.24
8	Accelerated ageing (48h)	217	17.71
9	Accelerated ageing (72h)	230	18.78

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

Seed Vigour traits	Desirable genotypes
Seedling length	IBYT-HI-17 (II), 2 nd GSBSN-28 (III), MGL-117 (III)
Seed density	IBYT-HI-17 (II), MBGSN 145 (VII), 2 nd GSBYT-02 (2015) (II), MGL-117 (III), IBON-HI-1 (2015-16) (V), IBON-HI-3 (2015-16) (V), IBON-HI-37 (2015-16) (V), INBON-15-16 (V)
Standard germination	UPB 1059 (I)
Seedling dry weight	IBYT-HI-17 (II), 2 nd GSBYT-02 (2015) (II), DWRB 101(I), 2 nd GSBSN-15-35 (VII), BH 13-26 (I), BH 15-17 (II)
Vigour index I	2 nd GSBSN-28 (III), MGL-58 (I), MGL-117 (III)
Vigour index II	IBYT-HI-17 (II), 2 nd GSBYT-02 (2015) (II), DWRB 101 (I), 2 nd GSBSN-15-35 (VII), BH 13-26 (I), BH 15-17 (II)
Electrical conductivity	IBYT-HI-13 (I), IBYT-HI-16 (I), IBYT-HI-18 (VI), IBYT-HI-15 (IV), HUB 242 (IV), 2 nd GSBYT-02 (2015) (II), DWRB 101(I), MGL 105 (I), IBON-HI-3 (2015-16) (V), 2 nd GSBSN-15-8 (VII), BH 13-20 (IV), BH 14-25 (V), BH 946 (V)
Accelerated ageing (48 h & 72 h)	IBYT-HI-19 (I), IBYT-HI-15 (IV), IBYT-HI-20 (I), MGL-64 (VI), DWRB 143 (I), BH 13-26 (I)

Values in parenthesis indicates cluster number

The mean performance of different clusters revealed that the clusters II and III were composed of highly vigorous genotypes because of their better cluster means for most of the traits studied. Therefore, clusters II and III might be considered desirable for selecting genotypes which may be used as promising parents for hybridization. The genotypes namely IBYT-HI-17, 2nd GSBYT-02 (2015), BH 15-17, RD 2909, 2nd GSBYT-23 (2015), 2nd GSBSN-28 (2015) and MGL-117 which fall in these clusters could be used in crossing programme to obtain high heterotic response and thus better segregants in subsequent generations.

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