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# EXPLORING THE GENETIC DIVERGENCE IN BARLEY STRAINS FOR SEED VIGOUR ATTRIBUTES

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## SUMMARY

Fifty diverse genotypes of barley were evaluated for different seed vigour parameters. Genetic diversity was assessed based on the observations for the different characters *viz*., seedling length (cm), seed density (g/cc), standard germination (%), seedling dry weight (mg), vigour index I & II, electrical conductivity (?S/cm/seed) and accelerated ageing test. All the genotypes were assembled into seven well defined clusters depending upon the similarity in the expression of their genetic divergence. Maximum number of genotypes were allocated in cluster III (13) followed by cluster II (12) and VII (6), while the cluster I was the smallest one with four genotypes only. Among the genotypes maximum distance within the same cluster was reported by cluster V (3.63) followed by cluster VII (3.37), cluster II (3.06) while the minimum intra-cluster distance was showed by the cluster IV (2.47). When diversity within clusters was studied it showed a range of 3.60 to 6.42. Cluster IV and V showed maximum inter-cluster distance of 6.42, followed by between cluster I and V (6.25). The lowest inter-cluster distance was observed between cluster II and III (3.60). Contribution of accelerated ageing (72 h) towards divergence was found maximum (37.80%) followed by seedling length (17.88%), electrical conductivity (17.63%), seedling dry weight (10.61%) whereas rest other traits contribute very little to divergence. Clusters III and V might be considered desirable for selecting genotypes which may be used as promising parents for hybridization. The genotypes which fall in these clusters were IBYT-HI-15, 2nd GSBYT-23 (2015), IBYT-HI-18, IBON-HI-67 (2015-16), AZAD, IBON-HI-13 (2015-16), DWRB 143, BH 13-26, 2nd GSBSN-60 (2015), INBON-15-16, MGL-62, 2nd GSBSN-15-35, IBYT-HI-20 and BH 959, RD 2909, MBGSN 145, 2nd GSBSN-28 (2015), BH 15-17 could be used in the hybridization programme for generation of wider variability.

**Key words :** Barley strains, seed vigour , genetic diversity

In India, barley is cultivated on about 0.69 million hectares with the production and productivity of 1.79 million tons and 2580 kg/ha, respectively. The average crop productivity of barley is highest in Punjab followed by Haryana, Rajasthan and Uttar Pradesh (Anonymous, 2017). To assure the safety of crop production, it is necessary to extend genetic background in crop breeding. It is well known that genetic diversity is the basis of biological diversity, and thus, it plays a key role in future breeding progress.

Evaluation and assessment of genetic diversity in crop species is fundamental for their improvement. The study of genetic diversity is the process by which variation among varieties or groups of individuals or populations is analyzed by a specific method or a combination of methods. In order to obtain a good insight into the available variation it is necessary to assess the level of variation between as well as within a representative set of breeding material. This information is also important for the maintenance and future use of the varieties. There are rare studies found in literature concerned to genetic diversity in barley based on seed vigour parameters while there are many records of studied conducted on morphological and yield attributing parameters. An effort is tried to make towards genetic diversity present among barley genotypes based on their seed vigour data.

The use of cluster analysis algorithms is an important strategy for classifying genotypes and analyzing genetic relationships among materials. In plant breeding programmes, assessment of the genetic diversity is done by the $D^2$ statistics devised by Mahalanobis in 1928. Now this technique is extensively used in variability related plant breeding studies. This statistical analysis has several advantages. First, it allows mixing of both qualitative and quantitative data and therefore all the available information on the sample can be utilized, cluster

analysis had been used in widely different fields, it can serve as a tool of selection and data reduction via similarity coefficient. Also, it provides useful information about genetic diversity in crops. Rao (1952) suggested the application of this technique for the assessment of genetic diversity in plant breeding.

## 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 (Department of Genetics & Plant Breeding), CCS Haryana Agricultural University, Hisar during *rabi* 2016-17 under late sown irrigated conditions. Each genotype was grown in three rows with a plot size of 3.0 x 0.69 $m^2$. Recommended package of practices were followed to raise the good crop.

The vigour potential of these seeds was computed by recording eight parameters viz. seedling length, seed density, standard germination, seedling dry weight, vigour index-I, vigour index-II, electrical conductivity and accelerated ageing test at 48 and 72 hours. All these vigour parameters were measured in three replications of each.

For calculation of standard germination, one hundred seeds of each genotypes, in three replications, were placed in between moistened rolled towel papers and kept at 20ºC in seed germinator. The final count was taken on 7$^{th}$ day and normal seedlings were considered for calculating per cent germination. Seedling length (shoot+ root), was measured on ten randomly selected normal seedlings taken from three replications each of standard germination and recorded in centimeters. Average of the ten seedlings was taken for final calculation. These ten seedlings whose length was measured, were dried in hot air oven for 24 hours at 80 ± 1ºC 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 in three replication of each genotype and weighed on electrical balance. These seeds were dipped in water having density of 1.0 at 20°C. Volume of water displaced by the seeds was recorded and seed density was calculated by using the following formula:

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

The seedling vigour indices were calculated as follows:

**Vigour Index-I**

Vigour index–I = Germination (%) x seedling length (cm)

**Vigour Index-II**

Vigour index–II = Germination (%) x seedling dry weight (mg)

Electrical conductivity measures the integrity of cell membranes. To measure the electrical conductivity, 100 healthy seeds in three replications were soaked in 50 ml deonized 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 ºC. The electrical conductivity of the seed leachates was measured by using a direct reading of conductivity meter. The conductivity was computed in µS/cm/seed.

For accelerated ageing test, seeds were placed in a single layer on the wire mess 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±1ºC different time intervals (48 h, 72 h) and tested for standard germination in three replications of 100 seeds each. The number of normal seedlings was counted on 7$^{th}$ day and expressed in percentage. Highly vigorous seeds are expected to tolerate high temperatures and humidity and retain their capability to produce normal seedlings in the germination test.

Genetic divergence among 50 genotypes was studied through Non-hierarchical Euclidean cluster analysis. Thereafter, promising genotypes were selected from each cluster which have higher mean than the general mean for different seed vigour traits Analysis of variance was executed to test the significance for each character as per the method given by Panse and Sukhatme (1967). Estimates of divergence were analyzed by using Mahalanobis (1936) $D^2$ statistics. The computation of $D^2$ values and for deciding group constellations, as per method recommended by Rao (1952) was followed. The dendrogram was constructed as per Ward's minimum variance method.

## RESULTS AND DISCUSSION

All the genotypes studied were significantly different for all the seed vigour parameters under investigation. It clearly indicates that the genotypes exhibited a wide range of variation and also suggested that experimental material was appropriate for further analysis.

The fifty barley genotypes were clustered into seven distinct groups. The distribution pattern of 50 genotypes in different clusters is presented in Table 1. The dendrogram shows the relation among the different genotypes used in the study based on seed vigour traits (Fig.1). The clusters were formed by grouping all the 50 genotypes in such a way that genotypes within each cluster had smaller $D^2$ value than those between clusters. The cluster membership profile revealed that cluster III emerged with highest number of entries as it was constituted by 13 entries followed by cluster II, VII having 12 and 6 varieties, respectively while Cluster I was the smallest one having only four genotypes namely IBYT-HI-19, IBYT-HI-13, 2nd GSBSN-15-8, MGL-117. The inter-cluster distance was higher than the intra-cluster, indicating wide genetic diversity among the genotypes (Table 2 and Fig. 2). A maximum inta-cluster distance was delineated by cluster V (3.63) followed by cluster VII (3.37), cluster II (3.06) while the minimum intra-cluster distance was showed by the cluster IV (2.47). When diversity within clusters was studied it showed a range of 3.60 to 6.42. Cluster IV and V showed maximum inter-cluster distance of 6.42, followed by between cluster I and V (6.25). The lowest inter-cluster distance was observed between cluster II and III (3.60). A lot of research work had been executed to select genetically diverse parents for hybridization in barley (Sharma *et al.* 2014, Hailu *et al*. 2016).

**Cluster means**

The seven well defined clusters showed considerable differences in mean values for the characters under study (Table 3). Maximum mean values of different seed vigour parameters for the seven clusters and most promising genotypes identified from different clusters were presented in Table 4 & 5 respectivily. Cluster I composed of four genotypes, exhibited maximum for standard germination, lowest for seed density, electrical conductivity and accelerated ageing (72 h), and had moderately high cluster means for remaining traits like seedling length, vigour index-I & II and accelerated ageing (48 h) except seedling dry weight which had low mean value. The best performing genotypes for electrical conductivity were IBYT-HI-13, 2nd GSBSN-15-8 and MGL-117. Cluster II comprised of 12 genotypes characterized by high mean value for seed density and moderately high values for rest other seed traits. The genotypes like IBON-HI-1 (2015-16), BH 13-20, BH 946 of this cluster showed best mean performance for the traits like seed density and accelerated ageing (72 h). Cluster III had 13 genotypes being largest one having characteristic features of highest value of accelerated ageing (48 h) and high mean values for the characters like seedling length, standard germination, vigour index-I and accelerated ageing (72 h) and low for electrical conductivity. The elite genotypes for traits such as seedling length, standard germination, vigour index-I were IBON-HI-67 (2015-16), AZAD, DWRB 143. Cluster IV, contained five genotypes recorded lowest mean values for most of the traits studied except for electrical conductivity and accelerated ageing (48 h & 72 h) for which, it showed high mean value. The best performing genotype was IBYT-HI- 16. Five genotypes constituted cluster V and characterized by high mean values for almost all traits studied except standard germination. Genotypes performed best for the traits such as seedling dry weight, vigour index-II were MBGSN 145, 2nd GSBSN-28 (2015), BH 15-17. Cluster VI contained five genotypes observed with low to moderate values for almost all the traits recorded except seedling dry weight which is noted with high mean value. Most desirable genotypes under this cluster were DWR 123, DWR 137, RD 2904. Cluster VII which consisted of five genotypes exhibited highest mean values for seedling length, vigour index-I and high for standard germination. Moderate to low for the traits like seedling dry weight, vigour index-II, electrical conductivity, seed density and accelerated

TABLE 1
Cluster membership profile of different genotypes

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

ageing (48 h & 72 h). Superior genotypes with desirable traits *viz.* Seedling length, vigour index-I were 2nd GSBYT-02 (2015), IBON-HI-37 (2015-16), K 560.

**Contribution of different traits towards divergence**

Contribution of accelerated ageing (72 h) towards divergence was found maximum (37.80%) followed by seedling length (17.88%), electrical conductivity (17.63%), seedling dry weight (10.61%) and accelerated ageing (48 h) which contributed 9.88% towards divergence whereas the remaining traits like seed density, standard germination and vigour index-I & II contributed very little to divergence. Poonia *et al*. (2017) in oat, Sharma *et al*. (2017) in wheat concluded with the similar results on seed vigour parameters.

The study showed wide variation from one cluster to another in respect of cluster means for eight characters, which indicated that varieties having distinctly different mean performance for various characters were reported into different clusters. Briggs and Dunn (2000) also reported a genetically diverse range of Western Canadian six-row barley cultivars differ in germination resistance and early seedling vigour. In the breeding programme the crossing between the entries belonging to cluster pairs having large inter-cluster distances and possessing high cluster means for one or other characters to be improved may be recommended for isolating desirable recombinants in the segregating generation in barley. Therefore, it can be concluded that clusters III and V might be considered desirable for selecting genotypes which may be used as promising parents for hybridization. The genotypes which fall in these clusters were IBYT-HI-15, 2nd GSBYT-23 (2015), IBYT-HI-18, IBON-HI-67 (2015-16), AZAD, IBON-HI-13 (2015-16), DWRB 143, BH 13-26, 2nd GSBSN-60 (2015), INBON-15-16, MGL-62, 2nd GSBSN-15-35, IBYT-HI-20 and BH 959, RD 2909, MBGSN 145, 2nd GSBSN-28 (2015), BH 15-17 could be used in the hybridization programme for generation of wider variability.

TABLE 2
Estimates of intra-and inter-cluster distances

| Clusters | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| I | **2.647** | 3.775 | 4.028 | 4.629 | 6.255 | 4.558 | 4.509 |
| II | | **3.057** | 3.604 | 4.193 | 4.978 | 4.121 | 4.289 |
| III | | | **2.702** | 4.745 | 4.653 | 4.078 | 4.299 |
| IV | | | | **2.468** | 6.415 | 4.657 | 6.250 |
| V | | | | | **3.632** | 4.029 | 4.780 |
| VI | | | | | | **2.728** | 4.480 |
| VII | | | | | | | **3.371** |

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

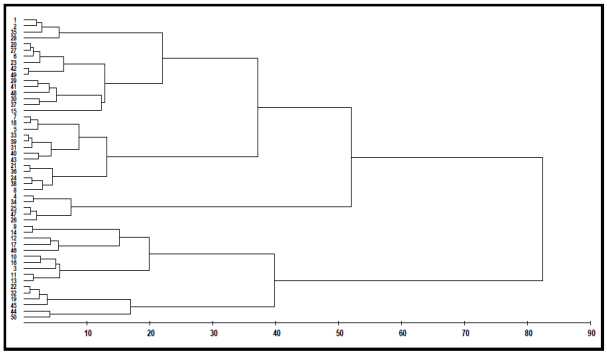
TABLE 3
Mean performance of different clusters for seed vigour traits

| Clusters/Characters | SL | SD | GP | SDW | VI | VII | EC | AA48 | AA72 |
|---|---|---|---|---|---|---|---|---|---|
| I | 30.15 | 1.05 | 98.17 | 125.04 | 2957.04 | 12276.68 | 0.39 | 80.83 | 33.33 |
| II | 31.12 | 1.36 | 96.17 | 126.65 | 2990.54 | 12200.54 | 0.44 | 81.61 | 59.08 |
| III | 34.00 | 1.15 | 97.05 | 134.78 | 3298.79 | 13080.53 | 0.41 | 89.80 | 74.62 |
| IV | 25.89 | 1.06 | 92.53 | 120.34 | 2393.56 | 11144.69 | 0.43 | 84.80 | 73.20 |
| V | 35.05 | 1.39 | 95.27 | 175.85 | 3338.96 | 16744.91 | 0.51 | 89.20 | 77.20 |
| VI | 30.17 | 1.16 | 94.87 | 172.53 | 2864.41 | 16379.5 | 0.45 | 82.80 | 63.07 |
| VII | 36.06 | 1.19 | 97.67 | 147.44 | 3522.17 | 14401.67 | 0.48 | 70.33 | 48.33 |

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

TABLE 4
Maximum mean values of different seed vigour parameters for the seven clusters

| Cluster No. | Seed Vigour parameters |
|---|---|
| I | Standard germination |
| II | High values of seed density |
| III | Accelerated ageing (48 h) |
| IV | High values of accelerated ageing (48 h & 72 h) |
| V | Seed density, seedling dry weight, vigour index-II, electrical conductivity, accelerated ageing (72 h) |
| VI | High values of seedling dry weight, vigour index-II |
| VII | Seedling length, vigour index-I |




Standarized Euclidean distance

Fig. 1. Dendrogram showing the clustering pattern of late sown barley genotype.

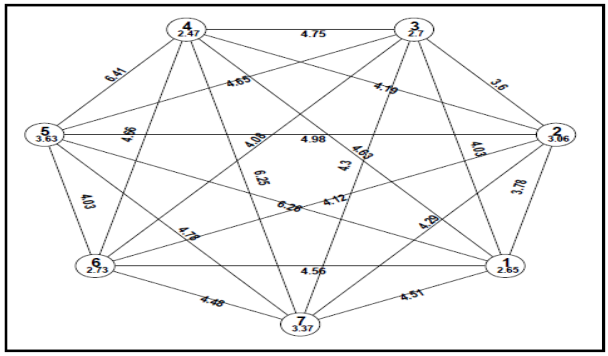



Fig.2. Mahalanobis Euclidean distances in late sown barley.

TABLE 5
Most promising genotypes identified from different clusters

| Cluster No. | Genotypes | Seed vigour parameters |
|---|---|---|
| I | IBYT-HI-13, 2nd GSBSN-15-8, MGL-117 | Electrical conductivity |
| II | IBON-HI-1 (2015-16), BH 13-20, BH 946 | Seed density, accelerated ageing (72 h) |
| III | IBON-HI-67 (2015-16), AZAD, DWRB 143 | Seedling length, standard germination, vigour index-I |
| IV | IBYT-HI- 16 | Electrical conductivity, accelerated ageing (48 h &72 h) |
| V | MBGSN 145, 2nd GSBSN-28 (2015), BH 15-17 | Seedling dry weight, vigour index-II |
| VI | DWR 123, DWR 137, RD 2904 | Seedling dry weight |
| VII | 2nd GSBYT-02 (2015), IBON-HI-37 (2015-16), K 560 | Seedling length, vigour index-I |

TABLE 6
Contribution of different characters towards divergence

| S. No. | Source | Times ranked 1st | Contribution towards divergence (%) |
|---|---|---|---|
| 1. | Seedling length (cm) | 219 | 17.88 |
| 2. | Seed density (g/cc) | 44 | 3.59 |
| 3. | Germination (%) | 19 | 1.55 |
| 4. | Seedling dry weight (mg) | 130 | 10.61 |
| 5. | Vigour index- I | 10 | 0.82 |
| 6. | Vigour index- II | 3 | 0.24 |
| 7. | Electrical Conductivity(µS/cm/seed) | 216 | 17.63 |
| 8. | Accelerated ageing test (48h) | 121 | 9.88 |
| 9. | Accelerated ageing test (72h) | 463 | 37.80 |

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