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MULTIVARIATE BIPLOT ANALYSIS OF EXOTIC BARLEY GENOTYPES BASED ON AGRO-MORPHOLOGICAL VARIABLES

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A panel of 141 barley genotypes was used for the current multivariate biplot analysis in order to quantify the genetic diversity for their meaningful utilization in barley improvement. The experimental material was evaluated in augmented design during crop season 2023-24 at Research Farm, CCS HAU, Hisar with a plot size of 1.5 m² occupied by each genotype. The results revealed high coefficient of variation for all the studied variables except of days to heading and maturity that explained low magnitude of variability. The association study among the variables exhibited significant positive correlation of grain yield with plant height, number of grains per spike, biological yield and harvest index. Principal Component Analysis identified first four components with more than one eigen value that cumulatively accounted for 72.637 percent of the total variation. The biplot analysis also displayed the positive association of grain yield with number of grains per spike, plant height, biological yield and harvest index as indicated by the acute angles between their vectors. The cluster analysis classified the genotypes into eight clusters with highest genetic distance among the genotypes of cluster VI. The genotypic clustering also illustrated maximum genetic distance between cluster III and VIII. Among all, cluster V characterized by higher grain yield and harvest index with long spikes. Furthermore, the biplot graph identified barley genotypes G 30, G 108, G 77 and G 71 as most promising and diverse genotypes for grain yield and G 77, G 50, G 97 and G 32 for biological yield. Hence, the study revealed the existence of enough genetic diversity among the genotypes tested that might be exploited for further genetic improvement of economic traits of barley. **ABSTRACT**

*Key words***:** Barley, Biplot analysis, Correlation, Clustering, PCA, Agro-morphological variables

Introduction

Barley (*Hordeum vulgare* L.) is a member of cereals and is primarily being utilized for livestock feed, human consumption and for malting purposes. In India, barley occupied an area and production of 0.62 million hectare and 1.69 million tonnes grain, respectively with productivity of 27.33 q/ha. Barley was cultivated on 15,300 hectares with a production of 53,300 tons in Haryana state that ranked second in average productivity (34.86 q/ha) after Punjab (36.54 q/ha) during 2022-23 (ICAR-IIWBR, 2023). This crop has given less importance for being many reasons, but yet there is an opportunity to obtain high yielding cultivars for competing with other crops. Breeding for yield enhancement through hybridization program is reliant on the presence of genetic diversity. The assessment of genetic diversity may serve as the reservoir of many novel traits conferring tolerance to different biotic and abiotic stresses. The planned utilization of genetically diverse genotypes permits suitable heterotic parental combinations in the segregating generations upon crossing. The diversity studies mainly rely on morphological, biochemical and molecular observations. Among them, morphological characterizations are the strongest determinants of the agronomic value of plants as these evaluations are direct, easy and do not require expensive technology (Bhandari *et al*., 2017). Genetic improvement of important agro-morphological traits of barley needs to be investigated for yield gain by exploring the inherent variability of these traits, their mutual association and their contribution towards diversity.

Multivariate analysis referred to evaluation of such data set where more than two variables are considered together. It facilitates the graphical representation of the underlying latent factors and interface between individual samples and variables. Principal component and cluster analysis are the multivariate techniques frequently used to group and characterize genotypes. The cluster analysis used to partition a set of data into clusters, where similar objects contained within the cluster, while dissimilar objects scattered in other clusters (Han *et al*., 2012). PCA used to reduce the dimensionality of large data sets into a smaller one that still contains most of the information in the large set (Jaadi, 2019). Biplot analysis is a multivariate analytical approach that graphically displays the 2-way data and allows visualization of the interrelations among genotypes and variables. Multivariate biplot analysis has been recommended for genetic diversity investigation in many crops.

Therefore, the present study was designed and conducted with a set of 141 barley genotypes employing multivariate biplot analysis aimed to identify component traits and to estimate the magnitude of genetic divergence for their utilization in future breeding programs.

Materials and Methods

A field trial was conducted during 2023-24 crop season at Research Farm, CCS Haryana Agricultural University, Hisarwith a set of 140 exotic barley genotypes along with one indigenous barley variety DWRB 137. This exotic barley material was received from ICARDA through Indian Institute of Wheat and Barley Research, Karnal. The experimental material under study comprised of70 two-rowed and 71 six-rowed barley including three check varieties *viz*., Rihane-03, V Morales and DWRB 137. Table 1 contained list of barley genotypes used in the current study along with codes assigned to them. Augmented Design was used to evaluate the experimental material. Each genotype was planted in paired rows of 2.5 m length spaced at 30 cm apart. The experimental material was sown on $12th$ November, 2023 and package of practices recommended were accordingly followed to raise the crop.

A total of tenagro-morphological traits *viz*., days to heading, days to maturity, plant height (cm), number of effective tillers per meter row, spike length (cm), number of grains per spike, 1000- grain weight (g), biological yield per plot (g), harvest index (%) and grain yield per plot (g) were recorded at appropriate stages of the crop growth. R software version 4.4.1 was used for statistical analysis of the recorded data.

Results and Discussion

The descriptive statistics of ten studied agro-

Table 1: List and clustering profile of exotic barley genotypes used in the study.

Code	Genotypes	RT	$\overline{\mathbf{C}}$
$\overline{G1}$	IBON-HI-24-1	$\overline{2}$	Ī
$\overline{G2}$	IBON-HI-24-2	$\overline{2}$	\overline{I}
$\overline{G3}$	IBON-HI-24-3	$\overline{2}$	Ī
G4	IBON-HI-24-4	$\overline{2}$	I
$\overline{G5}$	IBON-HI-24-5	$\overline{2}$	I
G ₆	IBON-HI-24-6	$\overline{2}$	I
$\overline{G7}$	IBON-HI-24-7	$\overline{2}$	I
$\overline{G8}$	IBON-HI-24-8	$\overline{2}$	$\mathbf I$
$\overline{G9}$	IBON-HI-24-9	$\overline{2}$	\overline{I}
$\overline{G10}$	IBON-HI-24-10	$\overline{2}$	$\bf I$
G11	IBON-HI-24-11	$\overline{2}$	$\rm I\hspace{-.1em}I$
G12	IBON-HI-24-12	$\overline{2}$	\mathbf{m}
$\overline{G13}$	IBON-HI-24-13	$\overline{2}$	$\overline{\mathbf{N}}$
$\overline{G14}$	IBON-HI-24-14	$\overline{2}$	$\rm III$
$\overline{G15}$	IBON-HI-24-15	$\overline{2}$	$\overline{\text{N}}$
$\overline{G16}$	IBON-HI-24-16	$\overline{2}$	$\rm III$
$\overline{G17}$	IBON-HI-24-17	$\overline{2}$	\mathbf{m}
G18	IBON-HI-24-18	$\overline{2}$	\mathbf{m}
$\overline{G19}$	IBON-HI-24-19	$\overline{2}$	$\overline{\text{V}}$
G ₂₀	IBON-HI-24-20	6	$\overline{\text{M}}$
$\overline{G21}$	IBON-HI-24-21	$\overline{6}$	$\overline{\text{VII}}$
$\overline{G22}$	IBON-HI-24-22	6	VІІ
$\overline{G23}$	IBON-HI-24-23	6	$\overline{\text{M}}$
G ₂₄	IBON-HI-24-24	\overline{c}	$\overline{\text{N}}$
$\overline{G25}$	IBON-HI-24-25	6	$\rm I\hspace{-.1em}I$
G ₂₆	IBON-HI-24-26	6	$\rm I\hspace{-.1em}I$
$\overline{G27}$	IBON-HI-24-27	6	$\rm I\hspace{-.1em}I$
$\overline{G28}$	IBON-HI-24-28	6	$\rm I\hspace{-.1em}I$
G29	IBON-HI-24-29	6	$\rm I\hspace{-.1em}I$
$G\overline{30}$	IBON-HI-24-30	6	$\rm I\hspace{-.1em}I$
$\overline{G31}$	IBON-HI-24-31	6	$\rm I\hspace{-.1em}I$
G32	IBON-HI-24-32	6	$\overline{\text{VIII}}$
$\overline{G33}$	IBON-HI-24-33	6	$\rm I\hspace{-.1em}I$
$\overline{G34}$	IBON-HI-24-34	$\overline{6}$	$\overline{\mathbb{I}}$
G35	IBON-HI-24-35	6	П
G36	IBON-HI-24-36	6	П
G37	IBON-HI-24-37	6	П
G38	IBON-HI-24-38	$\overline{2}$	V
$\overline{G39}$	IBON-HI-24-39	6	VІІ
G40	IBON-HI-24-40	\overline{c}	I
G41	IBON-HI-24-41	\overline{c}	$\overline{\text{N}}$
G42	IBON-HI-24-42	6	VШ
G43	IBON-HI-24-43	6	VI
G44	IBON-HI-24-44	6	$\overline{\text{M}}$
$\overline{G45}$	IBON-HI-24-45	$\overline{2}$	$\overline{\mathsf{V}}$
G46	IBON-HI-24-46	6	VI
G47	IBON-HI-24-47	\overline{c}	Ш
G48	IBON-HI-24-48	$\bar{2}$	$\mathbb N$

Continue Table 1...

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Continue Table 1...

morphological variables has been presented in Table 2. The results revealed enough genetic variability among the material evaluated as exhibited by wide range and higher coefficient of variation. The variables *viz*., biological yield, grain yield, number of tillers per meter row, number of grains per spike and plant height showed wider range. Similarly, number of grains per spike, harvest index, grain yield, number of tillers per meter row, biological yield and 1000-grain weight were recorded with

GS: Number of grains per spike; TGW: 1000- grain weight: BY: Biological yield per plot; HI: Harvest index; GY: Grain yield per plot; CV: Coefficient of variation

Table 2: Descriptive statistics of variables in barley.

high coefficient of variation. Days to maturity followed days to heading revealed low genetic variability. These findings are in congruence with earlier reports by Kaur *et al*., (2018).

The association analysis among the variables is depicted in Table 3. Both positive and negative trends were observed for correlation between the variables studied. The grain yield exhibited significant positive association with plant height, number of grains per spike, biological yield and harvest index. Some researchers also reported significant positive correlation of grain yield with biological yield per plant, harvest index and number of grains per spike (Ashok *et al*., 2024); and plant height (Kaur *et al*., 2018; Manhas and Kashyap, 2023). The positive and significant correlation were also observed for days to heading with days to maturity; number of tillers per meter row and plant height with spike length and biological yield; spike length with 1000-grain weight and biological yield; and number of grains per spike with harvest index. Some findings were in consonance with these results *i.e.* significant positive association of days to heading with days to maturity (Kaur *et al*., 2016; Aklilu *et al*., 2020); biological yield with number of effective tillers per plant and plant height (Hailu *et al*., 2016); biological yield with spike length (Abdullah *et al*., 2018); 1000-grain weight with spike length (Ashok *et al*., 2024).

Likewise, significant negative correlation was found for number of tillers per meter row with days to maturity and number of grains per spike; spike length with number of grains per spike; 1000-grain weightwith number of grains per spike; and biological yield with harvest index. These results were also corroborated for days to maturity with number of effective tillers per plant (Hailu *et al*., 2016); number of grains per spike with number of effective tillers per plant (Kumar and Sehrawat, 2021); number of grains per spike with spike length and 1000-grain weight (Hailu *et al*., 2016); and biological yield with harvest index (Ashok *et al*., 2024).

Principal Component Analysis (PCA) is a data reduction technique that reduced total number of variables into fewer ones that contribute to the maximum percentage of total variation. Additionally, PCA represents the association between all variables at once, is better criterion over correlation coefficient for assorting promising genotypes in different environments. PCA based on variable under study as depicted in Table 4 revealed that the first four components with eigen value > 1.00 accounted for about 72.637 percent of the total variation. The remaining PCs with an eigen value of < 1 were ignored due to their lesser contribution towards total variability. The first PC accounted for 22.742 percent of the total variance. Other PCs *i.e.* second, third and fourth explained 20.462, 15.652, 13.781 percent of the total

Variables	DM	TM	PH	SL	GS	TGW	GY	BY	н
DH	$0.713***$	-0.015	-0.124	-0.012	-0.134	0.039	-0.154	-0.088	-0.069
DM		$-0.18*$	-0.143	-0.039	0.010	-0.026	-0.049	-0.033	-0.020
IM			0.149	$0.221**$	$-0.49***$	0.091	0.036	$0.243**$	-0.116
PH				$0.32***$	-0.004	0.109	$0.210*$	$0.405***$	-0.132
SL					$-0.307***$	$0.272**$	0.051	$0.173*$	-0.065
GS						$-0.549***$	$0.308***$	0.052	$0.23**$
TGW							-0.131	-0.094	-0.012
\mathbf{G}								$0.398***$	$0.529***$
BY									$-0.516***$
***, **,* significant at 0.001 , 0.01 and 0.05 levels, respectively									

Table 3: Association among different variables in barley.

Variables/Components	PC1	PC2	PC ₃	PC ₄			
Days to heading	0.047	-0.469	-0.427	0.281			
Days to maturity	0.141	-0.400	-0.492	0.298			
Number of effective tillers per meter row	-0.416	0.046	0.056	0.081			
Plant height	-0.311	0.321	-0.207	0.134			
Spike length	-0.394	0.050	0.001	0.319			
No. of grains per spike	0.473	0.319	-0.223	-0.103			
1000-grain weight	-0.338	-0.217	0.291	0.276			
Grain yield per plot	0.107	0.476	-0.142	0.506			
Biological yield per plot	-0.298	0.346	-0.509	-0.110			
Harvest index	0.339	0.133	0.341	0.593			
Eigen value	2.274	2.046	1.565	1.378			
Proportion of total variation $(\%)$	22.742	20.462 15.652 13.781					
Cumulative percentage of variance	22.742		43.204 58.855	72.637			
PC: Principal Component							

Table 4: Loading of variables with respect to different principal components in barley

variability, respectively. Days to heading and maturity exhibited strong correlation with PC 2 & PC 3, respectively. Number of tillers per meter row, spike length, number of grains per spike and 1000-grain weight showed strong association with PC 1 whereas; plant height had high loading with PC 2. Grain yield and harvest index were highly associated with PC 4, however, biological yield indicated high loading on PC 3. In agreement with this study, Angassa and Mohammed (2021) reported 67.68% of the total variations cumulatively accounted by first two principal components while studied 138 barley accessions using nine quantitative traits. The relative contribution of various traits to the total variability has also been reported by Saroei *et al.,* (2017), Amezrou *et al*., (2018) and Kaur *et al*., (2018) in barley.

In biplot analysis, vectors of variables displaying acute angles are positively correlated while, those showing obtuse or straight angles are negatively correlated and the vectors with right angles represents no correlation.

Fig. 1: Biplot analysis of genotypes vis-a-vis variables.

Fig. 2: Dendrogram portraying clustering pattern of 141 barley genotypes (Refer to Table 1 for detail of genotypes).

The biplot was constructed in order to compare the relationship between genotypes and variables employing first two principal components that explained 43.20 percent of the total variation (Fig. 1). The biplot demonstrated positive association of grain yield with number of grains per spike, plant height, biological yield and harvest index as indicated by the acute angles between their vectors. Likewise, obtuse angles of days to heading and maturity, 1000-grain weight, spike length and number of tillers per meter row with grain yield

Clusters		П	Ш	${\bf I}$	V	VI	VШ	VШ	Genotypes in cluster
	1070.1								16
П	1491.1	1473.5							22
Ш	1967.1	1961.9	1130.5						17
\mathbf{I}	1121.3	1227.1	1713.2	874.9					21
v	1443.4	1390.5	1345.1	1144.9	777.6				10
$\mathbf{V}\mathbf{I}$	1794.6	1783.1	1663.8	1537.4	1167.1	1490.1			18
VШ	1532.9	1523.7	1466.3	1276.8	934.1	1290.2	1028.2		28
VIII	1624.8	2069.8	2551.7	1707.2	1992.3	2378.7	2115.3	1267.1	9

Table 5: Genetic distances between identified clusters.

Clusters	DH	DM	TM	PH	SL	GS	TGW	GУ	BY	н
	90	128	160	106	9.4	28	43.0	290	1629	18.2
π	97	136	104	98	8.3	66	35.4	373	1284	29.8
Ш	94	132	106	93	8.7	33	47.0	200	933	22.9
IV	96	135	143	101	9.3	29	44.6	261	1527	17.3
\mathbf{v}	93	132	142	94	9.7	32	45.7	380	1238	31.6
VI	95	133	93	89	7.5	64	33.7	243	1252	20.2
VП	90	130	100	99	8.3	72	34.7	353	1336	27.1
VШ	94	133	154	108	8.6	65	34.5	317	2060	15.1

Table 6: Performance of clusters for the studied variables.

showed negative correlation of these variables with grain yield. Similar kind of biplot approach has also been used by Abdullah *et al*., (2018) in barley for visualizing the association between variables. The distribution of the genotypes over the quadrants delineated the presence of significant genetic diversity among the studied barley genotypes. The distinctiveness of a genotype from other genotypes is measured by the vector length from their origin to the position of genotypes. The genotypes with long vector length may possess higher or extreme values for one or more variable and consequently their utilization in breeding program may respond heterotically. In addition, the variability explained by different variables can also be seen by the length of vectors pertaining to a particular variable. Long vectors denote the higher magnitude of variability and vice-versa. Similarly, the genotypes occupied position nearer to the origin of biplot performed stable performance compared to other ones for the studied variables.

The cluster analysis was also performed that categorized all the genotypes into eight distinct clusters as illustrated in Table 1. The clustering pattern identified cluster VII as largest one with twenty eight genotypes,

Table 7: Diverse and superior genotypes for different variables.

whereas, cluster VIII being smallest contained nine genotypes. Seventeen genotypes were grouped in cluster III with maximum genetic distance from cluster VIII (Table 5). Similarly, cluster V with ten germplasm deciphered minimum genetic distance from cluster VII. The results also revealed maximum intra-cluster distance for cluster VI with eighteen genotypes, implies the genotypes with relatively more diversity compared to genotypes belonging to other clusters. The results in addition also revealed minimum genetic distance among the genotypes of cluster V. Angassa and Mohammed (2021) also categorized 138 barley accessions into four clusters and reported wide range of diversity among studied barley accessions.

The average performance of genotypes in relation to different variables under study is portrayed in Table 6. The observations indicated the grouping of genotypes in cluster 1 found promising for days to heading, maturity and number of tillers per meter row. The genotypes of cluster III possessed highest 1000-grain weight among all clusters. The genotypes of clusters VI, VII and VIII performed better for plant height, number of grains per spike and biological yield, respectively. Similarly, cluster V characterized by higher grain yield and harvest index

with longest spikes. Several studies have also been conducted for assessment of genetic diversity in barley based on different agro-morphological characters for selecting genetically diverse genotypes for hybridization (Kaur *et al*., 2018; Kumar *et al*., 2024). The diverse and superior genotypes with desirable traits selected from the material tested are represented in Table 7. The genotypes G 30, G 108, G 77 and G 71 were found most promising and diverse for grain yield whereas, G 77, G 50, G 97 and G 32 performed better for biological yield. Similarly, the most diverse and desirable genotypes for 1000-grain weight and harvest index were G 101 and G 19, respectively.

Conclusion

The current study indicated existence of high genetic diversity among the exotic barley genotypes evaluated that may be exploited for development of potential genotype through suitable parental combinations. The association study reflected significant positive correlation of grain yield with plant height, number of grains per spike, biological yield and harvest index, could be considered as key components for yield determination. First four components identified by Principal Component Analysis cumulatively explained 72.637 percent of the total variation. The cluster analysis classified the genotypes into eight clusters of which the genotypes of cluster V characterized by higher grain yield and harvest index with long spikes, resultantly could be utilized as elite donor for barley improvement breeding. Among all genotypes, the most promising and diverse genotypes for grain yield were G 30, G 108, G 77 and G 71 while the genotypes, G 77, G 50, G 97 and G 32 performed better for biological yield. Multivariate with biplot analysis proved to be appropriate for diversity analysis intended to select the promising diverse genotypes for genetic improvement irrespective of crops.

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