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Agro-morphological Variability Study of Ethiopian Barley (*Hordeum vulgare* L.) Accessions for Their Important Agronomical Traits at Hadiya Zone, Southern Ethiopia

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Abstract: Utilization of conserved germplasm after assessing their level of diversity enables breeders by providing option of selection. Furthermore, exploiting existed genetic resources will enable us to increase production and secure food security in the era of climate change. In this regard 64 landrace barley accession and 3 released variety evaluated for eight quantitative traits in southern Ethiopia in 2019. The experiment was laid down in augmented block design with three standard checks which replicated in each block. The analysis of variance of eight quantitative traits indicated that there were significant differences ($P < 0.05$) between tested genotypes except for days to 75% maturity and plant height. Grain yield ranges from 20.72 to 57.33 quintals ha-1. The highest grain yield was harvested from Chefo (released variety). Nevertheless, the highest grain yield was measured from the one improved variety; 43 of the farmer's varieties were above the two improved verities. The principal component analysis resulted in two principal components (PC1 to PC2) with eigenvalues ranged from 1.74 to 4.30 containing variability of 21.80% and 53.77% respectively. The genotypes were broadly grouped into two distinct clusters. The first cluster contains 44 (65.67%) genotypes and the second cluster includes 23 (34.33%) genotypes including two of the improved varieties. Generally, the study showed the hidden potential of farmers' varieties accessions in improving yield through the utilization of conserved germplasm.

Keywords: Clustering, Diversity, Genotype, Farmers' Varieties, Principal Component

1. Introduction

Ethiopia is gifted with a range of diverse agro-ecologies arising from the wide ranges of variations in altitude from 110 m below sea level to 4620 m above sea level [1]. This complex topography and environmental heterogeneity together with the long-standing history and cultural diversity of the people in Ethiopia create scenarios suitable for and favoring the evolution and existence of a wide range of life forms.

The presence of extreme variable climatic and edaphic conditions in Ethiopia favors barley to be cultivated from 1400 to over 4000 meters above sea level [2, 3]. The long history of barley cultivation and the variety of agro-ecological zones and the variety of cultural practices have resulted in a country renowned for its large number of farmers' varieties and traditional agricultural practices [4]. Ethiopia is considered as a center of diversity of barley with the widest morphological diversity [2]. More genetic diversity was reported in Ethiopia than other countries of north Southwest Asia, the Middle East, North and Northeast Africa, and South Arabia [5].

Ethiopian barley farmer's varieties are important sources of valuable genes for several traits including barley yellow dwarf virus resistance, powdery mildew resistance, high lysine content, vegetative vigor, drought tolerant and resistance to several barley diseases [6]. Besides, they have useful agronomic future such as good tillering, tolerance to marginal soil, resistance to barley shoot fly, aphids and frost resistance, vigorous seedling establishment and rapid grain filling period [7].

The existence of genetic diversity has paramount significance for improving productivity and maintenance of diversity in a country like Ethiopia [8]. Barley can tolerate more adverse growing environments such as drought or lower soil fertility than wheat, it has some production constraints. The main production constraints of barley in Ethiopia are unpredictable rainfall pattern is the main along with a poor supply of improved seed, untimely supply of inputs like fertilizers [9]. Barley grain yield is affected by agronomic traits like, number of spikes per plant, number of kernels per spike and thousand seed weight that each contributes directly and indirectly to final grain yield [10].

More than 15,000 barley accessions have been maintained in the gene bank of the Ethiopian Institute of Biodiversity until 2014 [11]. A current report indicated that the accessions reached about 17000 accessions collected from barley growing regions of the country [12]. In addition, the most of collected and conserved barley accessions are not yet studied for their important agronomic traits. To make a wise decision on how to utilize and conserve the available barley genetic resource, studies have to be done on the genetic diversity of the crop [11].

Therefore characterizing this huge genetic resource is not a work we put on the table for tomorrow while our farmer is still straggling with low yielder varieties. Hence, the study aimed to assess the preliminary performance of barley accessions and genetic variability associations for yield and yield related agro-morphological traits of barley accessions.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted at Wachemo University research station in Hadiya Zone of Southern Nation Nationalities Peoples Region during the main rainy season. Geographically, the experimental station is located at 7°32'44"N latitude and 37°52'50"E longitude and an altitude of 2270 meters above sea level.

2.2. Experimental Material and Design

Sixty four farmers' barley accessions were obtained from the Ethiopian Biodiversity Institute genebank and three standard checks (*Chefo, Awedo and Bira*) were obtained from locally grown barley verities in the study area included in the study (Table 1).

The experiment was laid down in augmented block design with no replication among the barley accessions and three standard checks repeated in every block. The accessions were sowed on 9 August 2018 in the main cropping season with diammonium phosphate (DAP) at the rate of 100 kg ha^{-1} and UREA at the rate of 100 kg ha^{-1} (complete application at sowing) and the other management practices were applied as per recommended for the research site. The gross plot size was 2 m x 0.8 m (1.6 m^2). Each plot accommodated four rows of 2 m length with distance of 20 cm between rows. The outer rows at both ends of plots were considered as borders. The two middle rows were designated as sampling area. 0.5 m and 1 m distance kept between plots and blocks respectively.

Table 1. *List of barley accessions and standard checks used for the study.*

S.N	Accession Number	S.N	Accession Number	S.N	Accession Number	S.N	Accession Number
1	242576	18	244945	35	239528	52	243311
2	240790	19	244775	36	244925	53	245126
3	236140	20	244769	37	243568	54	244930
4	241685	21	243589	38	243566	55	242094
5	238825	22	242056	39	244888	56	243611
6	238642	23	239527	40	243555	57	244936
7	244938	24	242097	41	245122	58	242067
8	244939	25	244767	42	244779	59	244931
9	236139	26	241677	43	242571	60	242575
10	239520	27	239526	44	242062	61	243609
11	244927	28	242063	45	243588	62	243179
12	243304	29	243190	46	244923	63	242061
13	243559	30	243192	47	243308	64	244932
14	244941	31	243579	48	244774	65	Chefo
15	244934	32	243309	49	243580	66	Awedo
16	244933	33	242066	50	242095	67	Bira
17	244770	34	244778	51	244929		

2.3. Data Collection

All field and laboratory data were recorded according to barley descriptor list [13] based on plant based and plot based traits. Data for plant height (PH), spikelet per spike (SPS), kernel per spike (KPS) and spike length (SL) were recorded based on randomly selected and tagged 20 individual plants from each plot. Data for day to 50% flowering (DF), days to 75% maturity (DM), thousand seed weight (TSW), and grain yield (GY) were taken from the whole row for each accession and grain yield per plot converted into hectare for the analysis.

2.4. Statistical Analysis

2.4.1. Analysis of Variance

Analysis of variance was analyzed for all quantitative

traits using R statistical software (version 4.0.5; augmented RCBD package).

2.4.2. Principal Component Analysis

The data were standardized to mean zero and variance of one before computing principal component analysis to avoid differences in measurement scales. The principal component based on the correlation matrix was calculated using R statistical software (version 4.0.5; Facto Mine R package).

2.4.3. Euclidean Distance and Clustering of Genotypes

Euclidean Distance (ED) was computed from all traits after standardized as established by Sneath and Sokal [14]. The distance matrix from phenotype traits was used to construct dendrogram based on the Unweighted Pair Group Method with Arithmetic Means (UPGMA). The results of the cluster analysis were presented in the form of dendrograms. R statistical software (version 4.0.5; factoextra package) used for the analysis of distance matrix and constructing Dendrogram. The optimum number of clusters was determined by the gap stat Method using R statistical software version 4.0.5).

3. Results and Discussion

3.1. Analysis of Variance

The analysis of variance of eight quantitative traits under study indicated that there were significant differences (P <0.05) between tested genotypes except for days to 75% maturity and plant height (Table 2). Thus, the study showed that the presence of variability for the character considered which can be exploited for further barley improvement program through selection breeding programs.

The genotypes had a range of 56 to 87 days to 50% flowering and 88 to 127 days to 75% maturity (Table 3). The two genotypes 242061 and 243611 were genotypes that attain 50% early within 57 days. But genotypes 239526, 242062 and 243609 were delayed for 50% flowering. The presence of a wide range of maturity within the genotypes will allow breeders to develop varieties for short growing season areas and extended rainfall season areas. Report indicated reported that day to 50% flowering of 20 Ethiopian barley genotypes ranges from 75 to 100 days [15].

Table 2. *Mean square values of eight quantitative characters of 64 barley accessions along with the three standard checks.*

Source of Variation &	Mean Square							
Degree freedom	DF	DM	PH	SPS	KPS	SL	TSW	GY
Block (3)	1.64^{ns}	100.31^{ns}	9.29^{ns}	1.07^{ns}	1.21ns	0.12^{ns}	0.91^{ns}	41.98^{ns}
Genotypes (66)	56.48**	207.61^{ns}	39.42^{ns}	114.42**	19.03**	0.99**	25.68**	83.45*
Accessions (63)	49.83**	176.73^{ns}	32.32^{ns}	107.36**	109.19**	1.00**	24.09**	55.46
Checks (2)	74.08**	545.08*	272.16**	543.07**	641.63**	2.08**	112.25**	967.69**
Checks vs Accessions (1)	541.90^{ns}	2342.10**	1.63^{ns}	291.81**	306.58**	2.96**	71.41**	26.94^{ns}
Error (6)	4.97	102.64	17.06	7.26	7.38	0.05	1.64	21.67
CV	3.40	9.70	5.10	5.10	8.80	2.60	3.00	12.90

*, **, ns, Significant at (p< 0.05 and (p < 0.01), and nonsignificant respectively. DF= days to 50% flowering; DM= days to 75% maturity; PH= plant height (cm); SPS=number of spikelet per spike; KPS = number of kernels per spike; SL= spike length (cm); TSW = thousand seed weight (g); GY = grain yield (quintals/ha); CV, coefficient of variation (%).

Table 3. *Minimum, maximum and mean values of eight quantitative traits of 67 barley genotypes.*

Traits	Min	Max	Mean
Days to 50% flowering	56.00	87.00	65.25
Days to 75% maturity	88.00	137.00	104.26
Plant height (cm)	65.50	97.80	80.92
Spikelet per spike	20.90	57.40	31.60
Kernel per spike	20.05	56.75	30.90
Spike length (cm)	6.40	10.70	8.41
Thousand seed weight (g)	29.60	50.80	42.31
Grain yield (quintal/ha)	20.72	57.33	36.04

Regarding the number of spikelets per spike, number of kernels per spike and spike length were highly significant (P< 0.01) variations observed between tested genotypes. The value ranges from 20.9 to 57.4, 20.05 to 56.75 and 6.4 to 10.07cm for number of spikelets per spike, number of kernels per spike and spike length respectively. The highest and the smallest number of spikelets per spike and number of kernels per spike were recorded from 244779 and 242066 respectively. The spike length of genotypes ranges from 6.4 to 10.7cm with a mean of 8.41cm. A wide range of spike lengths (3.82 to 9.38 cm) were reported from Ethiopian barley genotypes by Ebrahim et al. [15].

Thousand seed weight ranges from 29.6 to 50.8 g with a mean of 42.3 g and report revealed that the thousand grain weight of 18 Ethiopian barley accessions ranged from 21.2 to 52.7 g with a mean of 36.2 g [16]. A wide range of variation was also observed among examined genotypes in grain yield. The yield ranges from 20.72 to 57.33 quintals ha^{-1}. The highest grain yield was harvested from *Chefo* (released variety) and the lowest yield was from the landrace accession 239527. Even though the highest grain yield was measured from the one improved variety; 43 farmer's varieties were able to produce higher grain yield than the two improved varieties. This show that the hidden potential of landraces in improving yield through the utilization of conserved germplasm. In addition, the presence of such wide variation in yield will help in the development of barley variety. Since increasing the production of grain yield is the ultimate goal of plant breeding. Report showed that the grain yield of Ethiopian Barley genotypes ranged from 22.58 to 62.02 quintals ha^{-1} [15].

3.2. Multivariate Analysis

3.2.1. Principal Component Analysis

The principal component analysis (PCA) was used for the

reduction of the data set and to transform the available data set into principal components. In this study using eight phenotypic quantitative traits PCA analysis was performed and presented in table 4. PCA transformed eight data set into eight factors loadings with the pattern illustrated that the first principal component (PC1) contributed the highest variability and the last principal component (PC8) contributed the lowest variability, which accounted for the entire (100%) variability.

The principal component analysis result indicated that the first two principal components (PC1 to PC2) with eigenvalues ranged from 1.74 to 4.30 containing variability of 21.80% and 53.77% respectively and totally the first two PC contain total variability of 75.57% (Table 4). These PC1 and PC2 had eigenvalue more than 1 [17]; while the rest (PC3 to PC8) of the PCS had eigenvalue less than 1 [18], and would not be considered in the interpretation of the results obtained due to that they were not significantly influencing and contributing to the variability among the barley genotypes.

In the PC1 kernel per spike (0.91), spike length (0.91), thousand seed weight (-0.87), and spike length (-0.85) respectively contributed high variability with positive and negative loading compared to the rest traits. The PC2 accounted for 21.80% of the total variation and was mainly influenced by grain yield (0.83) and plant height (0.69) with positive loading. Therefore, most of the variations among genotypes in PC1 and PC2 were brought due to these major traits indicated above.

Table 4. *Principal component factors, eigenvalues, individual, and cumulative variability of eight quantitative traits of 67 barley genotypes.*

	Factor loadings							
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**	**Factor 7**	**Factor 8**
Days to 50% flowering	0.71	-0.44	0.47	0.05	0.07	-0.07	0.24	0.00
Days to 75% maturity	0.78	-0.35	0.37	0.24	0.13	0.03	-0.24	0.00
Plant height (cm)	0.03	0.69	0.66	-0.23	-0.18	-0.01	-0.05	0.00
Spikelet per spike	0.91	0.32	-0.15	-0.13	0.10	0.16	0.03	0.03
Kernel per spike	0.91	0.32	-0.14	-0.12	0.10	0.16	0.03	-0.03
Spike length (cm)	-0.85	-0.04	0.18	-0.33	0.37	-0.02	-0.02	0.00
Thousand seed weight (g)	-0.87	-0.20	0.26	0.17	-0.03	0.33	0.05	0.00
Grain yield (quintals/ha)	-0.26	0.83	0.04	0.45	0.17	-0.06	0.06	0.00
Eigenvalue	4.30	1.74	0.93	0.49	0.24	0.17	0.13	0.00
Variability (%)	53.77	21.80	11.63	6.06	3.02	2.13	1.57	0.02
Cumulative (%)	53.77	75.57	87.20	93.27	96.29	98.41	99.98	100.00

Biplot analysis was carried out based on the first two PCs to visualize associations of traits and their contribution to the total variability (Figure 1). The genotypes that were positioned on the right top quadrant were closely associated and characterized by having the highest number of spikelets per spike and the highest plant height. The genotypes found on the top left quadrant were characterized by the highest grain yield. Furthermore, the biplot put the genotypes on the left bottom quadrant that have the highest spike length and thousand seed weight. Genotypes positioned on the right bottom are also characterized by late flowering and maturity. The genotypes positioned around the origin had similar genetic characteristics, while the genotypes that were found far from the origin are separated from the rest of the group due to their peculiar genes and considered as unrelated genotypes. Hence, selection of these genotypes as potential parents would result in successful hybridization to develop heterotic groups in the breeding programme (Figure 1).

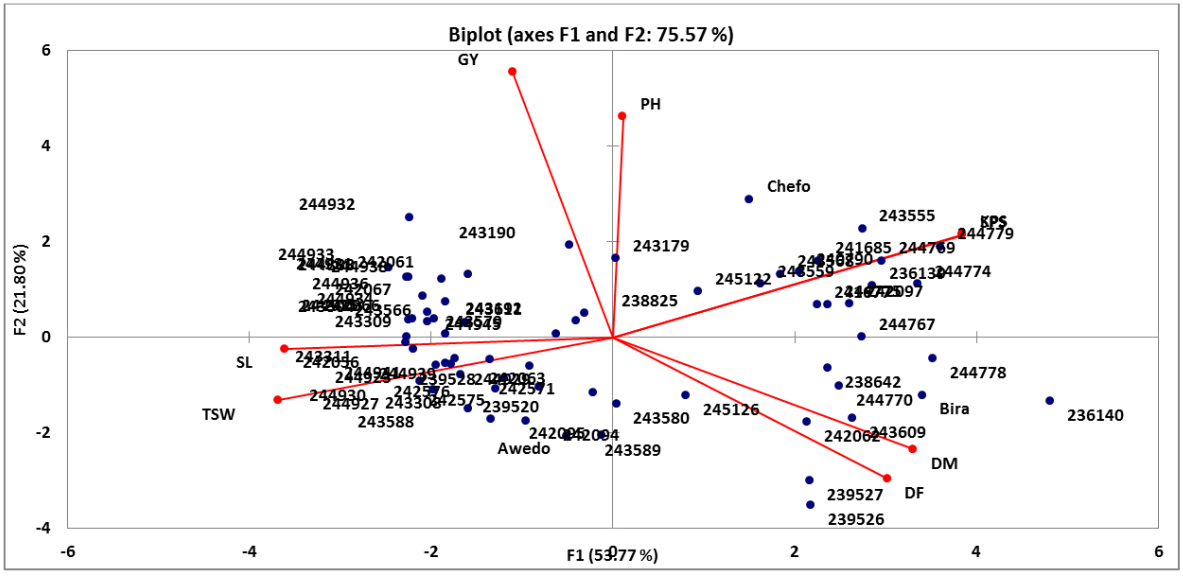

Figure 1. *Biplot (axes PC1 and PC 2) of eight quantitative traits of 67 barley genotypes.*

3.2.2. Cluster Analysis

The optimum number of clusters was determined by the gap stat method using R statistical software version 4.0.5 (Figure 2). The distance matrix from eight quantitative traits was used to construct dendrograms based on the Unweighted Pair Group Method with Arithmetic Means (average) (Figure 3).

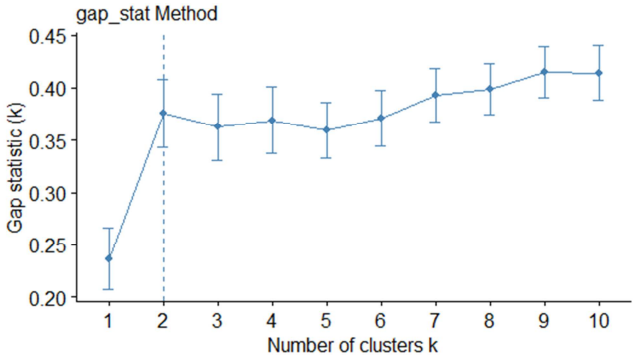

Figure 2. *Determination of the optimum number of clusters using gap_stat method.*

The Euclidean distances of all possible pairs of 67 barley genotypes were estimated by Euclidean distance from eight quantitative traits and the results as Euclidean distance matrix. The Euclidean distances of the genotypes ranged from 0.43 to 8.38. The highest Euclidean distance (8.38) was computed between accessions number 244932 and 36140 accessions. Whereas, the smallest Euclidean distance was estimated between accessions number 243311 and 242056 (Figure 3).

The genotypes were broadly grouped into two distinct clusters. The first cluster contains 44 (65.67%) genotypes including one improved variety (standard check). The second cluster was also constructed by 23 (34.33%) genotypes including two of the improved varieties (standard check) (Table 5; Figure 3). Similar results have been reported by after examining a different number of farmers' varieties of barley accessions and categorized them into a different number of clusters based on their morphological traits [12, 19, 20].

The constructed dendrogram showed that the existence of variability among the studied barley genotypes. Characterization of such genotypes and clustering them based on their morphological traits and genetic similarity will help in the identification of best performer parents for hybridization/ crossing. Grouping of genotypes by using multivariate analysis based on their similarity in the present study would be valuable for barley breeders in that the most important accessions in the population may be selected from different clusters for barley improvement programs.

Table 5. *Clusters, number of genotypes, and list of genotypes in each cluster of evaluated 67 barley genotypes.*

Cluster	Number of genotypes	List of Genotypes
I	44	242576, 238825, 244938, 244939, 239520, 244927, 243304, 244941, 244934, 244933, 244945, 243589, 242056, 242063, 243190, 243192, 243579, 243309, 242066, 239528, 244925, 243566, 244888, 245122, 242571, 243588, 244923, 243308, 243580, 242095, 244929, 243311, 245126, 244930, 242094, 243611, 244936, 242067, 244931, 242575, 243179, 242061, 244932, Awedo
II	23	240790, 236140, 241685, 238642, 236139, 243559, 244770, 244775, 244769, 239527, 242097, 244767, 241677, 239526, 244778, 243568, 243555, 244779, 242062, 244774, 243609, Chefo, Bira

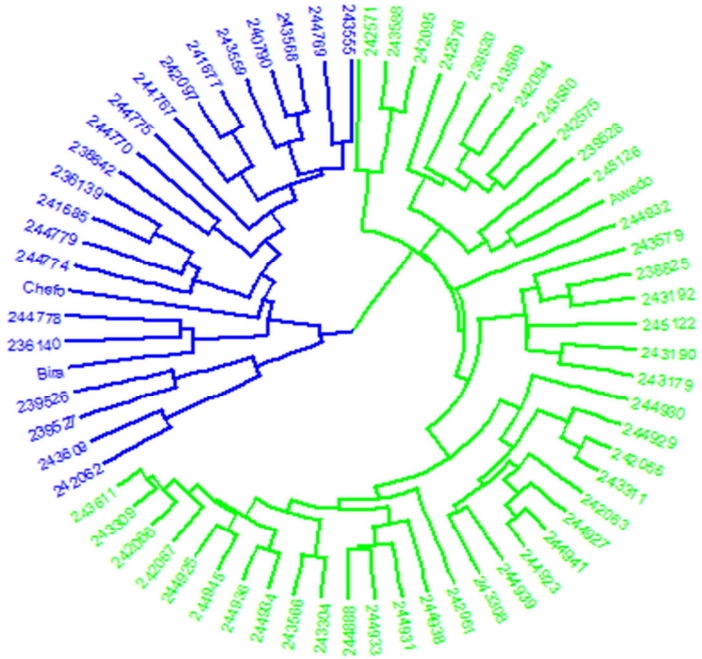

Figure 3. *Dendrogram of barley genotypes based on eight quantitative traits using Unweighted Pair Group Method with Arithmetic Means (average).*

Genotypes clustered in the first cluster were characterized by having greater grain yield, spike length and thousand grain weights. Contrary, genotypes clustered in the second cluster took the highest days for 50% flowering and 75% maturity. Besides, genotypes in this cluster were characterized by greater plant height and number of spikelets per spike (Table 6; Figure 4).

Table 6. *Cluster means value for eight quantitative traits of 67 barley genotypes grouped in two clusters.*

Traits	Cluster I	Cluster II
Days to 50% flowering	61.15	70.68
Days to 75% maturity	96.10	114.86
Plant height (cm)	80.47	81.66
Spikelet per spike	24.50	43.42
Kernel per spike	23.67	42.92
Spike length (cm)	8.99	7.49
Thousand seed weight (g)	45.56	36.96
Grain yield (quintal/ha)	36.87	33.92

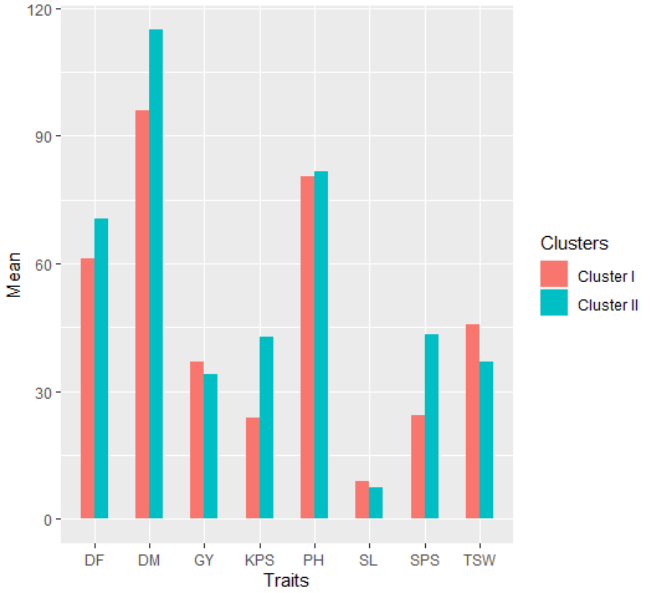

Figure 4. *Cluster mean values of two clusters for eight quantitative traits of 67 barley genotypes.*

4. Conclusion

Sixty four farmers' varieties accessions and three standard checks were evaluated for their agro-morphological variability using augmented design. The analysis of variance showed that there were significant differences between tested genotypes except for days to 75% maturity and plant height. The principal component analysis revealed that the first two PC with eigenvalues ranged from 1.74 to 4.30 containing variability of 21.80% and 53.77% respectively which contain total variability of 75.57%. The cluster analysis grouped the genotypes were broadly into two distinct clusters. The first cluster contains 65.67% genotypes and the second cluster includes 34.33% genotypes including two of the improved varieties. Generally, the study clearly showed that the potential of farmers' varieties for traits considered in the study. Hence, the accessions were divergent and have great genetic diversity which could be used for further barley breeding programs to increase productivity barley variety and enhance food security in the country. Since the experiment was done at a single location and used morphological traits to examine the diversity of the barley genotypes, it is recommended to conduct the experiment at different locations and include molecular tools to assess the diversity of the genotypes for important agronomic traits. In addition, it is also recommended to further evaluate the promising genotypes at multiple locations and different years to obtain more reliable information.

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