Current Journal of Applied Science and Technology

39(36): 102-110, 2020; Article no.CJAST.62184
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Evaluation of Genetic Divergence among Barley Genotypes Based on Agro-Morphological Characters

Zerihun Jalata[1*], Belay Garoma[1] and B. C. Nandeshwar[1]

[1]Department of Plant Sciences, College of Agriculture, Shambu Campus, Wollega University, Ethiopia.

Authors' contributions

This work was carried out in collaboration among all authors. Author ZJ designed the study, performed the statistical analysis, wrote the protocol and wrote the draft of the manuscript. Authors BG and BCN managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i3631078
Editor(s):
(1) Dr. Michael Ignatius Ferreira, Western Cape Department of Agriculture, South Africa.
Reviewers:
(1) Goutam Paul, University of Engineering and Management, Kolkata, India.
(2) Sanjeeva Rao, ICAR-Indian Institute of Rice Research, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62184

Original Research Article

Received 17 August 2020
Accepted 23 October 2020
Published 03 December 2020

ABSTRACT

The knowledge of nature and the magnitude of divergence existing in the breeding materials are useful to identify suitable parents or populations to combine favorable genes. Thus, 28 barley genotypes were evaluated at Gitilo site in RCB design with three replications during the 2018/19 season. The aim of the study was to investigate the magnitude of genetic divergence among the existing breeding materials. The result revealed that the barley genotypes were grouped into four clusters. The inter-cluster distance was greater between clusters I and II, followed by cluster II and III and then between clusters II and IV, I and IV, III and IV, between I and III so that crossing among parents from distant clusters result in wide array populations with desirable alleles. Besides this, cluster mean analysis showed clusters IV and III contained desirable characters for high yield potential including net blotch and scald resistance indicating their suitability for direct variety development. The variation studied through principal component analysis revealed four principal components (PC1:32.7%, PC2:22.4%, PC3:16.7% and PC4:11.6%) accounting for about 83.4% of the total variation. Furthermore, the biplot graph identified barley genotypes or populations 21, 20, 24 and 12 as desirable parents mainly for grain yield, biomass yield per plant and thousand kernel weights.

**Corresponding author: E-mail: jaluu_z@yahoo.com;*

Keywords: Biplot; cluster analysis; cluster mean; principal component analysis.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the first crop plants to be domesticated, remains one of the most important crops used for human consumption and animal feed worldwide [1]. It is among the major staple crops in Ethiopia covering a large area (about one million hectares) and its national productivity is about 2.0 tons ha^{-1} [2,3]. While world barley productivity is about 3.0 tons ha^{-1} [2] showing a large yield gap. Thus, the development of high yielding potential populations and/or varieties is necessary to increase the yield through suitable parental combinations.

The divergence between crops has been extensively identified and used in the improvement of crop species in modern plant breeding as it may serve as the reservoir of many novel traits conferring tolerance to different stresses [4]. The assessment of genetic diversity permits to select the genetically diverse parents to obtain the desirable recombinant in the segregating generations upon crossing. Thus, the inclusion of more diverse parents is believed to increase the chances of obtaining stronger heterosis and gives a broad spectrum of variability in segregating generations [5]. Genetic diversity study methods rely on mainly morphological, biochemical, and molecular (DNA-based) data [4,6]. Among this, morphological characterizations are the strongest determinants of the agronomic value and taxonomic classification of plants. Furthermore, morphological evaluations are direct, inexpensive, easy, and do not require expensive technology [4].

To estimate the degree of divergence between biological populations at the genotypic level and to assess the relative contribution of different characters to the total divergence, multivariate analysis employing Mahalanobis's D^2 statistic has been used as a powerful tool. This analysis provides the basis for grouping the germplasm collection into different more or less homogenous groups and therefore helps in reducing the size of germplasm collection to be evaluated [7]. The Mahalanobis distance is a measure of the distance between two points in the space defined by two or more correlated variables. While the cluster analysis method assumes discontinuities within the data. It depicts the pattern of relatedness between genotypes based on evolutionary relationships or phenotypic performance. It is used to group similar lines/germplasm in one group and differentiate other groups [4].

Principal component analysis (PCA) is another multivariate method that has been also used to classify genotypes. PCA is a data reduction technique applicable to the quantitative type of data. PCA transforms multi-correlated variables into another set of uncorrelated variables for further study. These new sets of variables are linear combinations of original variables. It is based on the development of eigenvalues and mutually independent eigenvectors (principal components) ranked in descending order of variance size or declining information content. Such components give scatter plots of observations with optimal properties to study the underlying variability and correlation [4].

The barley materials selected for this study were composed of useful lines and/or varieties with the diverse origin and visually vary in their morphological and agronomic characters which are potentially useful for breeding programs to generate genetic variability or develop improved variety or varieties. However, the extents of genetic divergence or variability existing among these breeding populations were not studied. Thus, this study would give a better overview of the barley populations about the extent of divergence present between populations or genotypes, the desirable parents and traits, the main traits contributing more to total divergence, and the major traits that should be focused on for indirect selection to improve yield. Therefore, the study was aimed to estimate the magnitude of genetic divergence existing among barley genotypes or populations for future breeding programs.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the 2018/19 main cropping season at Gitilo research site, Shambu Campus, Wollega University which is about 325 km west of Addis Ababa, Ethiopia and 9 km west of Shambu town. The experimental site is found at Gitilo Dale, located at 09°32'N to 037°03'E with an altitude of 2795 meters above sea level. The climate of the area is high land (2500-3500 m) and receives an annual rainfall of 1650-1780

mm. The area is mainly barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) growing area [3].

2.2 Planting Materials and Design

Twenty eight barley genotypes were field evaluated at Gitilo site, Ethiopia. The experiment was laid in a randomized complete block design with three replications and seeds of each genotype or populations were sown in four rows of 2.5 m length and 0.40 m width at 15 cm between plants during the 2018/19 main cropping season.

2.3 Data Collected

Data was taken on ten randomly selected plants from each plot for recording on days to heading, days to maturity, grain filling period, plant height, number of effective tillers per plant, spike length, number of kernels per spike, thousand kernel weight, biomass weight per plant, grain yield per plant and harvest index per plant, scald and net blotch disease.

2.4 Statistical Analysis

All data measured on 13 agro-morphological traits were subjected to analysis of variance using Proc GLM procedures of SAS version 9.0 [8]. The multivariate analysis provides the basis for grouping the germplasm collection into different more or less homogenous groups which help reduce the size of the germplasm collection to be evaluated. The agglomerative (starting with single elements and aggregating them into clusters) hierarchical clustering based on the Unweighted paired group method using the arithmetic mean (UPGMA) method was applied using the Proc CUSTER program of SAS [8]. UPGMA method provides more accurate grouping information on breeding materials used following pedigrees and calculated results found most consistent with known heterotic groups than the other clusters [4]. The data for all quantitative traits were standardized to mean zero and variance of one before clustering to avoid the difficulty of different scales that may have arisen due to differences in measurement scales.

The genetic distance between clusters was calculated using the generalized [9] statistics as.

$$D^2_{(ij)} = (\bar{X}_i - \bar{X}j) S^{-1} (\bar{X}_i - \bar{X}j)$$

Where, D^2ij = the square distance between any two genotypes i and j; Xi and Xj = the vectors for the values for genotypes i^{th} and j^{th} genotypes; and S^{-1}= the inverse of pooled variance-covariance matrix within groups. The D^2 values obtained from pairs of clusters were considered as the calculated values of Chi-square (χ2) and were tested for significance at 1 and 5% probability levels against the tabulated values of χ2 at p degrees of freedom, where, p is the number of characters considered (p = 13) [10].

2.5 Principal Component Analysis

The general formula to compute scores on the first component extracted in a principal component analysis was:

PC1=b11(X1) + b12 +···b1p= (XP) using R-software version R-3.4.0 [11],

Where, PC1= the subject's score on principal component 1 (the first component extracted), b1p = the regression coefficient (or weight) for observed variable p, as used in creating principal component 1 and XP = the subject's score on observed variable p. Using R software the loadings of the genotypes and the traits were determined to clarify the association among principal components and traits, principal components and genotypes, genotypes and their traits and the different agronomic traits. In addition, the biplot graph was constructed from PC1 and PC2 to display the association of the different agronomic traits and genotypes.

2.6 Contribution of Individual Characters

The character contribution towards genetic divergence was computed by using the method given by Singh and Chaudhary [12]. Considering all the combinations, each character is ranked based on di= y^j_i-y^k_i Where, di = mean deviation, y^{jj} = mean value of j^{th} genotype for the i^{th} character, y^{jk} = mean value of k^{th} genotype for the i^{th} character, The character having the highest mean difference be ranked 1 and subsequently lowest mean difference be allotted rank p, where the p - the number of characters/traits.

3. RESULTS AND DISCUSSION

ANOVA showed that there was a highly significant ($p<0.01$) difference among all barley

genotypes for all characters studied (data not shown) indicating the presence of variability among barley genotypes for agro-morphological characters. In the present study, 28 barley genotypes were clustered into four clusters (Table 1; Figure 1) based on D^2 values. The maximum number of genotypes were included in Cluster I (10 genotypes) followed by cluster IV (7), cluster III (6), and cluster II (5).

3.1 Intra and Inter-Cluster Distances

The average intra- and inter-cluster divergences among barley genotypes studied were of varying magnitude (Table 2). The result revealed that there were highest inter-cluster distances between cluster I and II (163.79**) followed by between cluster II and III (92.53**) and then between cluster II and IV (65.24**), cluster I and IV (61.22**), cluster III and IV (25.01**) and cluster I and III (17.99**) (Table 2). Furthermore, the intracluster D^2 values were larger than in clusters III and IV (3, 08) followed by clusters I and II (2.77) (Table 2) which indicates their more homogeneity. Generally, the result suggests larger inter-cluster distance revealing a high amount of genetic diversity among the genotypes studied and so that selection of diverse parents with desirable traits from divergent clusters more likely produce wide variability with desirable segregants. Thus, hybridization between selections from cluster I with II, II with III, II with IV, III with IV, and I with III are, therefore, expected to produce relatively better genetic recombination or heterotic hybrids and maximum segregation in their progenies. However, the average intracluster distance was lower than the inter-cluster clusters (Table 2). The crossing of genotypes belonging to the same cluster is not expected to yield desirable segregants.

Table 1. Distribution of 28 barley genotypes into different clusters

No.	Cluster	Cluster member	Genotype
1	I	10	Sabini (1), Grace (2), Misrach (3) Miscal-21 (5). Agegnehu (7), Sabini/ Grace (8), Sabini/Misrach (9), Sabini/Miscal-21 (11), Sabini/Agegnehu (13), Grace/Miscal-21 (16)
2	II	5	HB1307 (4), Grace/Misrach (14), Grace/Agegnehu (18), HB1307/ Agegnehu (25), Sabini/HB1307 (10)
3	III	6	Grace/HB1307 (15), HB42/Agegnehu (28), Misrach/Agegnehu (22), HB1307/Miscal-21 (23), Misrach/HB1307 (19), Misrach/Miscal-21 (20)
4,	IV	7	HB42 (6), Sabini/HB42 (12), Grace/HB42 (17), Misrach/HB42 (21), HB1307/HB42 (24), Miscal-21/HB42 (26), Miscal-21/Agegnehu (27)

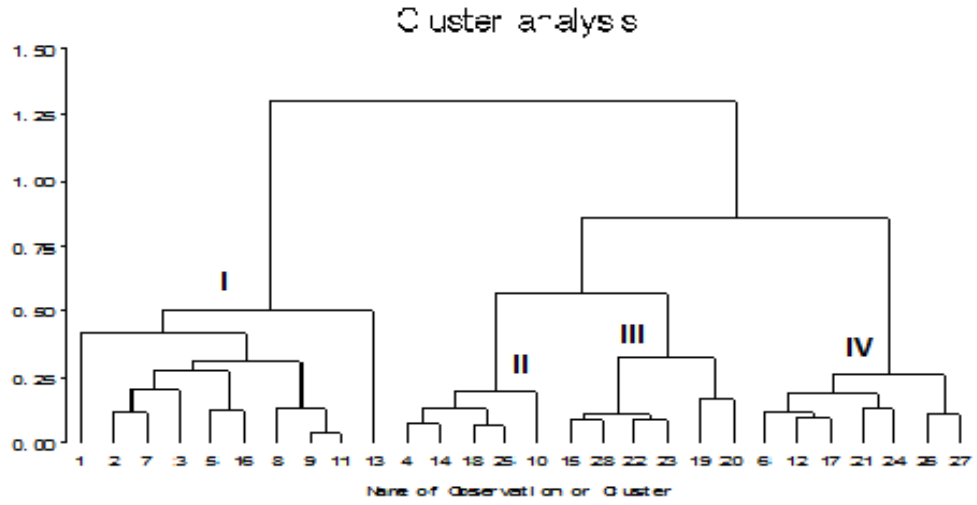

Figure 1. Dendrogram showing the clustering of 28 barley genotypes into groups

Table 2. Average Intra (bold) and inter-cluster distance (D^2) values for 28 barley genotypes

Cluster	I	II	III	IV
I	2.27	163.79**	17.99**	61.22**
II		2.77	92.53**	65.24**
III			3.08	25.01**
IV				3.08

Populations geographically separated for a longer period are expected to be genetically distant naturally from one another when compared with populations subjected to the same environmental pressure due to micro-evolutionary factors [13]. In the present study, the distribution of genotypes having a diverse origin and agro-morphological characters were grouped in the same cluster (I) showing that the geographical diversity was not fully reflected in the genetic diversity as these genotypes, i.e. Sabini and Grace (introduced from abroad), Misrach (pure line selection from Acc. Kulumsa 1/88 and released from Debre Brehan center), Miscal-21 (introduced from ICARDA/CIMMYT and released from Holeta center), Agegnehu (selection from Acc.218950-08 and released from Sirinka center). Diversity results also from the interaction of farmer management practices and ecological or geographic factors to determine population structure which affects the distribution of allelic variation within and between populations [14]. The cluster analysis grouped the genotypes into clusters that exhibit high homogeneity within a cluster and high heterogeneity between clusters [15].

Similarly Shrimali et al. [7] studied on 30 barley genotypes under both normal and moisture stress environments on and grouped them into seven clusters. Ahmad et al. [16] also studied 133 barley accessions using 14 morphological characters and clustered into seven clusters. Furthermore, Seid et al. [17] clustered 20 barley cultivars into three different clusters using agro-morphological traits. Abebe et al. [18] also studied 199 accessions and obtained high morphological variation. Tesfahun et al. [19] studied on 100 barley genotypes and grouped into four cluster groups for Asasa and six clusters at Ambo locations. While at both locations, genotypes showed maximum differentiation on days to maturity, grain filling period, tillers per plant, and spike per plant. Tigist et al. [20] report on199 barley accessions showed high morphological variation within regions and altitudes in Ethiopian and the clustering of accessions did not show grouping based on regions of origin.

The comparison of cluster means for characters under study showed remarkable genetic differences between the clusters for various characters (Table 3). Thus, both cluster III and IV had specifically better resistance to net blotch (mean range of 49.63 to 113.40) and scald (mean range of 494.90 to 157.11) diseases. In addition to this, cluster IV had a higher grain yield per plant (32.97 g), many kernels per spike (43.89), thousand kernel weight (56.83 g), and biomass yield per plant (97.10 g); Moreover cluster III showed gave higher grain yield per plant (33.95 g) and harvest index per plant (41.08). While cluster I tend to shorter in plant height (95.91 cm), early in maturity (23.54), and larger in spike length (8.84 cm), but we're more susceptible to net blotch (123.57) and scald (1188.79) diseases, less number of kernels per spike (27.47), less biomass yield (68.01) and grain yield per plant (27.32) than other clusters. Therefore, the cluster means analysis suggests that clusters IV and III contained desirable yield characters which can be considered valuable for direct variety development.

3.2 Principal Component Analysis

Principal component analysis (PCA) simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components [21]. PCA is interpreted based on the correlations among variables: the further the number from zero in either direction, the greater the positive or negative correlation [22]. PCA provides variable independence and balanced weighting of traits, which leads to an effective contribution of different characters based on respective variation [23]. In this study, the variation studied through PCA revealed that the first four principal components (with the value of PC1: 32.7%, PC2: 22.4%, PC3: 16.7, and PC4: 11. 6%) having eigenvalues greater than 1 are contributing about 83.4% of the total variation (Table 4). The sign of the loading indicates the direction of the relationship between the components and the variable. According to Chahal and Gosal [24] characters with a lower absolute value closer to zero influence the clustering less than those with the largest absolute value closer to unity within the first principal component.

Accordingly, first Principal Component One (PC1) had positive component loading from biomass yield per plant (0.378) followed by plant height (0.336), days to maturity (0.313), grain yield (0.298), number of kernels per spike (0.211), thousand kernel weight (0.193) and

maximum negative loading for scald (-0.371) followed by harvest index per plant (-0.164), net blotch (-0.127) (Table 4). The characters which load positively or negatively contributed more to the diversity and they were the ones that most differentiated the clusters. Similarly, Ahmed et al. [16] report of the first five principal components having greater than 1 eigenvalue contributed more than 83.40% genetic variation. And the PC1 accounted for 32.7% of the total variation as well as the characters contributing more positively to PC1 coincides with this study.

The major contributing characters for the diversity in the second principal component (PC2) were thousand kernel weight (0.440) followed by spike length (0.400), number of kernels per spike (-0.381), number of effective tillers per plant (0.380), and plant height (0.270) (Table 4). Similarly, the characters which load positively or negatively in PC3 and PC4 (Table 4) contributed more to the diversity and they were the ones that most differentiated the clusters, Therefore, PC1 accounted for the largest contribution to total genetic diversity. Some potentially important traits have been identified which is positively correlated with PCA and these can be exploited for specific trait improvement. Another study indicated characters such as thousand kernel weight, plant height, days to head, and days to maturity accounted for variation and played role in differentiating accessions collected from different regions and altitude classes into principal components [20].

3.3 Biplot Analysis

A biplot was depicted to visualize the association among principal components and characters, principal components and genotypes, genotypes and their characters, and among the different agronomic characters as shown in Figure 2. Thus, barley genotypes 21, 20, 24 and 12 had the largest score in the first PC and located in the positive direction on this PC which indicated that the genotypes were highly correlated with characters that have the highest positive loading in PC1 like grain yield per plant, biomass yield per plant, days to maturity, thousand kernel weight, grain filling period, plant height and effective tillers per plant. Thus, the genotypes have better performance in these agro-morphological traits than other genotypes. Moreover, there was also a close association between grain yield per plant and biomass yield per plant, between days to heading and number of kernels per spike, between thousand kernel weight and effective tillers per plant, between plant height and grain filling period. On the other hand, the grain filling period and net blotch contributed less to total genetic diversity (short arrows) (Figure 2).

Moreover, the performance of genotypes for any character could be determined by the arrow direction and position of the genotype. Hence, the barley genotypes which showed better performance for different characters include; genotype 21 showed higher in days to maturity (135) and grain yield per plant (58.5 gm), genotypes 27 and 19 showed higher mean value for days to heading (79.3, 81.3 respectively); genotypes 26 and 22 for many kernels per spike (55.6, 50.0 respectively);, genotype 20 for both plant height (108.1); and biomass yield per plant (101.1) (between both arrows), genotypes 4,9 and 13 showed better performance for spike length (8.9, 9.6, 9.7 respectively).

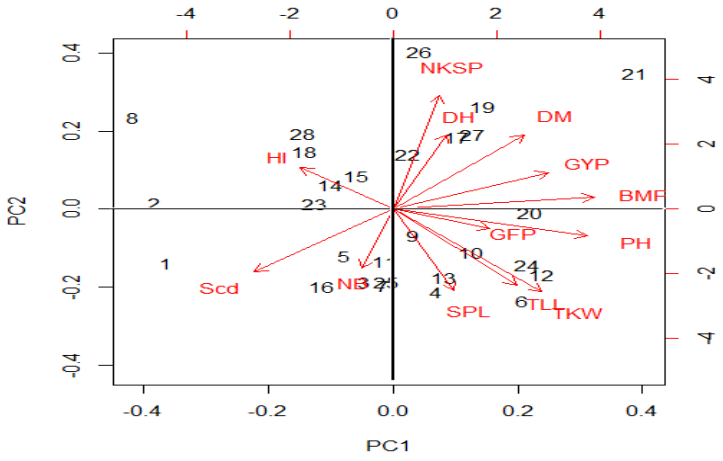

Figure 2. Principal component analysis biplot of barley genotypes and their characters

Table 3. Cluster means for 13 different characters of 28 barley genotypes

Cluster	NB	Send	DH	GFP	DM	PH	ETL	SPL	NKSP	TKW	BMP	GYP	HI
I	123.57	1188.79	76.23	47.31	123.54	95.91	17.28	8.84	27.47	49.69	68.01	27.32	40.73
II	105.38	821.74	75.02	48.08	123.08	102.66	18.96	8.34	35.86	51.84	83.26	31.36	37.32
III	49.63	494.90	75.83	50.12	125.95	99.98	18.57	7.35	41.33	46.42	82.32	33.95	41.08
IV	113.40	157.11	81.43	48.29	129.71	111.09	18.30	8.70	43.89	56.83	97.10	32.97	33.63
Mean	101.94	716.64	77.23	48.29	125.5	101.78	18.12	8.40	36.05	51.12	81.07	30.88	38.43

NB =Net blotch, Scd= Scald, DH=days to heading, GFP=grain filling period, DM=days to maturity, PH=plant height, ETL=effective tillers/plant, SPL=spike length, NKSP=number of kernels spike^{-1}, TKW=thousand kernel weight, BMP=biomass yield plant^{-1}, GYP=grain yield plant^{-1} and HI=harvest index

Table 4. Principal components loadings and their % contribution to the total divergence of different barley agro-morphological characters

Characters	PC1	PC2	PC3	PC4	% Contribution to total divergence
Net blotch (NB)	-0.127	0.204	0.088	-0.396	16.2
Scald (Scd)	-0.371	0.142	0.087	0.316	66.2
Days to heading (DH)	0.162	-0.183	-0.549	0.162	0.8
Grain filling period (GFP)	0.173	0.129	0.475	-0.209	0.5
Days to maturity (DM)	0.313	-0.127	-0.320	0.045	0.7
Plant height (PH)	0.336	0.270	0.114	0.026	2.4
Effective tillers per plant (TLL)	0.153	0.380	0.147	0.097	0.9
spike length (SPL)	-0.001	0.400	-0.366	0.227	0.0
Number of kernels per spike (NKSP)	0.211	-0.381	0.172	0.053	2.0
Thousand kernel weight (TKW)	0.193	0.440	-0.132	-0.030	1.7
Biomass yield per plant (BMP)	0.378	0.144	0.111	0.236	5.0
Grain yield per plant (GYP)	0.298	0.027	0.228	0.485	2.2
Harvest index per plant (HI)	-0.164	-0.211	0.221	0.473	1.2
Eigenvalue	3.33	2.22	1.56	1.07	
Variance explained (%)	32.7	22.4	16.7	11. 6	
Cumulative (%)	56.03	70.84	81.23	88.37	

For harvest index per plant, better performance was observed for genotypes 8(34.7%), 28(43.9%), 18(41.7%), and 14(38.0%).

For thousand kernel weight and effective tillers per plant, better performance was observed for genotypes 10(63.9, 19.7), 12(67.1, 24.5), 6(64.8, 23.6), and 24(66.1, 19.0), respectively. Genotype 1(339.3, 1179.5) showed very susceptible to both net blotch and scald diseases, respectively, while genotype 5(138.1), 16(105.1) and 25(74.5) showed highly susceptible to net blotch disease (Figure 2). The distance between the 14, 15, 18, 23, and 28 genotypes on the biplot graph was small which was may be mainly attributed to the harvest index as this character is the nearest trait to these genotypes. Most of the agro-morphological characters were negatively associated with harvest index as well as scald and net blotch diseases as they are located in opposite direction and far apart from each other. Genotypes 1, 2 and 8 were located far left on PC1 in the negative direction indicating poor performances in many several agronomic characters (Figure 2). Therefore, a multivariate technique of grouping genotypes in this study has practical applications in breeding for improving yield potential.

3.4 Percent Contribution of Each Character towards Total Divergence

The contribution of different plant characters for genetic divergence is important for further selection and choice of parents for hybridization. The highest contribution in the manifestation of genetic divergence was exhibited by scald (66.2%) followed by net blotch disease (16.2%), biomass yield per plant (5.0%), plant height (2.4%), grain yield per plant (2.2%) and many kernels per spike (2.0%) and thousand kernel weight(1.7%). While the rest characters had a negligible contribution towards total divergence (Table 4). Similarly, Shrimali et al. [7] reported under limited moisture condition, biological yield per plant contributed maximum towards total genetic divergence.

4. CONCLUSION

There exists high genetic diversity among barley genotypes tested which is useful to combine favorable alleles for desirable agronomic traits. The genotypes were grouped in four clusters and there was a high inter-cluster distance than intracluster. Hence, the improvement can be achieved by the hybridization of diverse parents with desirable traits between clusters I and II, II and III, II and IV, III and IV as well as I and III to develop a dynamic population. And cluster IV and III had desirable yield and yield-related traits which show the possibility of direct selection for variety development. The principal component analysis had grouped the estimated barley genotypes into four main components (PC1: 32.7%, PC2: 22.4%, PC3: 16.7 and PC4: 11. 6%) together contributing 83.4% to the total variation. The biplot graph identified barley genotypes 21, 20, 24 and 12 as desirable parents mainly for grain yield, biomass yield per plant, and thousand kernel weights.

ACKNOWLEDGEMENTS

We are grateful to Wollega University for their financial support in conducting this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zemede A. Barleys of Ethiopia. Genes in the field on-farm conservation of crop diversity. Stephen B. Brush, Lewis Publishers. 2000;77-107.
2. FAOSTAT. Food and Agriculture Organization of the United Nations Statistics Division. World Production of Barley Grain; 2016.
Available:http://faostat3.fao.org/home/E.date
(Accessed 20 July 2016)
3. CSA (Central Statistics Authority). The Federal Democratic Republic of Ethiopia, Central Statistical Agency Agricultural Sample Survey 2016/ 2017 (2009 E.C). Report on Area and Production of Major Crops (Private Peasant Holdings, *Meher* season). Statistical Bulletin No. 584, Addis Ababa. 2017;1:118.
4. Bhandari HR, Bhanu AN, Srivastava K, Hemantaranjan A. Assessment of genetic diversity in crop plants: An overview. Advances in Plants and Agricultural Research. 2017;7(3):279–286.
DOI: 10.15406/apar.2017.07.00255
5. Ananda IJ, Murty BR. Genetic divergence, and hybrid performance in linseed. Indian Journal of Genetics and Plant Breeding. 1968;28:178-185.

6. Mahesh M, Sudhanshu J, Bhadoria VS. Genetic divergence studies in soybean (*Glycine max* L. Merrill). International Journal of Agriculture Sciences. 2017;9(19):4196-4197.
7. Shrimali J, Shekhawat AS, Kumari S. Genetic divergence in Barley (*Hordeum vulgare* L.) under normal and limited moisture stress conditions. International Journal of Current Microbiology and Applied Science. 2017;6(8):2220-2226.
8. SAS (System Analysis Software). SAS Statistical Software Version NC, USA. 9.1; 2004.
9. Mahalanobis PC. On the generalized distance in statistics. Proc Nat Inst Sci India B. 1936;2(1):49-55.
10. Urdan TC. Statistics in plain English. 2nd Ed. Lawrence Erlbaum Associates, Mahwah, New Jersey, USA; 2005.
11. R Software. R Software Version 3.4.0 (2017-04-21), the R Foundation for Statistical Computing Platform: I386-w64-mingw32/i386 (32-bit); 2017.
12. Singh RK, Chaudhry BD. Biometrical methods of quantitative genetic analysis. *Kalyani* Publishers, Ludhiana, India. 1985;30-34.
13. Chandel KPS, Joshi BS. Multivariate analysis in green-seeded pea. Indian Journal of Agricultural Science. 1983;53(4):198-200.
14. Ramanatha Rao V, Hodgkin T. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue, and Organ Culture. 2002;68:1–19.
15. Jaynes DB, Kaspar TC, Colvin TS, James DE. Cluster analysis of spatiotemporal corn yield pattern in an Iowa field. Agronomy Journal. 2003;95:574-586.
16. Ahmad Z, Ajmal SU, Munir M, Zubair M, Masood MS. Genetic diversity for morpho-genetic traits in barley germplasm. Pakistan Journal of Botany. 2008;40(3): 1217-1224.
17. Seid Ebrahim, Eleni Shiferaw, Faris Hailu. Evaluation of genetic diversity in barley (*Hordeum vulgare* L.) from Wollo high land areas using agro-morphological traits and Hordein. African Journal of Biotechnology. 2015;14(22):1886-1896.
18. Abebe TD, Bauer AM, Léon J. Morphological diversity of Ethiopian barley (*Hordeum vulgare* L.) with geographic regions and altitudes. Hereditas. 2010;147: 154–164.
19. Tesfahun Alemu, Luiz Antônio dos Santos Dias, Robson Fernando Missio. Genetic divergence among barley accessions from Ethiopia. Crop Breeding and Applied Biotechnology. 2010;10:116-123.
20. Tiegist Dejene, Bauer AM, Léon J. Morphological diversity of Ethiopian barley (*Hordeum vulgare* L.) with geographic regions and altitudes. Hereditas. 2010;147: 154–164.
21. Ajmal SU, Minhas NM, Hamdani A, Shakir A, Zubair M, Ahmad Z. Multivariate analysis of genetic divergence in wheat (*Triticum aestivum*) germplasm. Pakistan Journal of Botany. 2013;45(5):1643-1648.
22. Mumtaz AD, Saeed HM, Arshad M, Yousaf MI. Estimation of genetic diversity in sorghum genotypes of Pakistan. J. Natn. Sci. Foundation Sri Lanka. 2018;46(3): 271-280.
23. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants—Salient statistical tools and considerations. Crop Science. 2003;43:1235–1248.
24. Chahal GS, Gosal SS. Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science International. 2002;604.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62184