

Traits Diversity Analysis of Malt Barley (*Hordeum vulgare* L.) Genotypes under Irrigation at Koga of West Gojjam in Ethiopia

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Abstract

Barley is one of the most highly cultivated crops in Ethiopia. The assessment of genetic diversity using quantitative traits is of prime importance in many contexts, particularly in differentiating well defined populations. The aim of this study was to select superior malt barley genotypes that meet the yield and quality standards for malting purposes. Forty nine malt barley genotypes including two checks were tested at koga of west Gojjam in Ethiopia under irrigation in a 7 × 7 simple lattice designs with two replications during off season of 2013. Using estimated D² values 49 genotypes were grouped into nine clusters with maximum genotypes (20) in cluster I and (14) in cluster II. Principal component analysis for malt barley genotypes revealed that the first four principal components accounted for more than 68.3% of the variation explained by explanatory variables. Agronomic characters having relatively higher value in the first four principal components had more contribution to the total diversity and they were responsible for the differentiation of the nine clusters. Nonetheless, considering the tremendous variability observed among the genotypes, further testing of these genotypes in different localities is suggested.

Keywords: Genetic diversity; Malt barley; Yield genotypes

Introduction

Barley (*Hordeum vulgare* L.) is one of the five major crop species of the world which is widely used for stock feed, human food and malting. In the year 2009, the global barley production was estimated over 150 million tons harvested from 54.13 million hectares of which Africa contributed over 4.99 million tons harvested from 4.7 million hectares [1].

Barley is one of the most highly cultivated crops in Ethiopia, with more than 1,700,000 metric tons produced on 1,046,000 hectares in 2010 [2]. It is one of the most important cereal crops, mainly grown by smallholder farmers at mid- and high-altitudes in North West Ethiopia, predominantly between 2200-3000 m above sea level [3]. Eighty percent of the production is in the Oromia and Amhara regions. ORDA (2008a) estimated that about 15,945 tons of malting barley is produced annually in Ethiopia [4].

The assessment of genetic diversity using quantitative traits is of prime importance in many contexts, particularly in differentiating well defined populations. Several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives, of which Mahalanobis's generalized distance occupy a unique place and an efficient method to gauge the extent of diversity among genotypes, which quantify the differences among several quantitative traits [5].

Ethiopia has a shortage of malt barley to meet the demand of the local [7]. Consequently, the breweries demand for malt is met through imports, which account for 69% of the total annual requirement [8]. While there is immense potential for producing malt barley in Ethiopia, its production is restricted to a few areas, most importantly the Arsi-Bale area. This had led to shortages of malt supply for the ever-growing local breweries. Currently, malt barley is grown mainly in South and North Gondar and Awi zones of Amhara region. Recently, its trials are also showing a promise that malt barley can also be grown under irrigation, and it is grown mainly for seed purpose under irrigation in areas like Koga. This has necessitated the expansion of malt barley production to other potential areas, including the Amhara region to satisfy the ever-increasing demand for raw materials by the beverage industry, and to ensure dependable and higher cash returns to the farmers. The present study has been undertaken with 49 malt barley

genotypes to understand the nature and the characters contributing genetic diversity by D² analysis.

Materials and Methods

Description of the study area

The Experiment was conducted in Amhara National Regional State Mecha Woreda, Koga irrigation site of Adet research area using irrigation during 2012/2013 dry season. It located between 11°10' to 11°25' N and 37°02' to 37°17' E. It receives an average annual rain fall between 1000 to 2000 mm. The site is situated at an altitude of 1960 meters above sea level and the climate is classified as Woiyna-dega. The dominant soil type of the area is nitosols.

Experimental material

The experimental material consists of 49 malt barley genotypes including one standard check (Miscal-21) and one local check (Fire-gebis). These genotypes were obtained from Adet Agricultural Research Centre.

Experimental design and layout

The experiment was laid out in a simple lattice design (7 × 7) with two replications. Each genotype were grown in 4 rows having a plot length of 2.5 m with a spacing of 20 cm between the rows or 2 m² plot size, spacing of 40 cm between plots, 1.5 m between blocks and 2 m between replications was maintained (Figure 1). Seeds were hand drilled at a rate of 85 kg/ha. According to Adet Agricultural Research Center recommended fertilizer rate (50 kg/ha urea and 100 kg/ha DAP) was used. The two middle rows of the experimental plots were taken for recording the observations.

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Received April 29, 2017; Accepted May 11, 2017; Published May 18, 2017

Citation: Tilahun A, Alemu F (2017) Traits Diversity Analysis of Malt Barley (*Hordeum vulgare* L.) Genotypes under Irrigation at Koga of West Gojjam in Ethiopia. Mol Biol 6: 190. doi: [10.4172/2168-9547.1000190](https://doi.org/10.4172/2168-9547.1000190)

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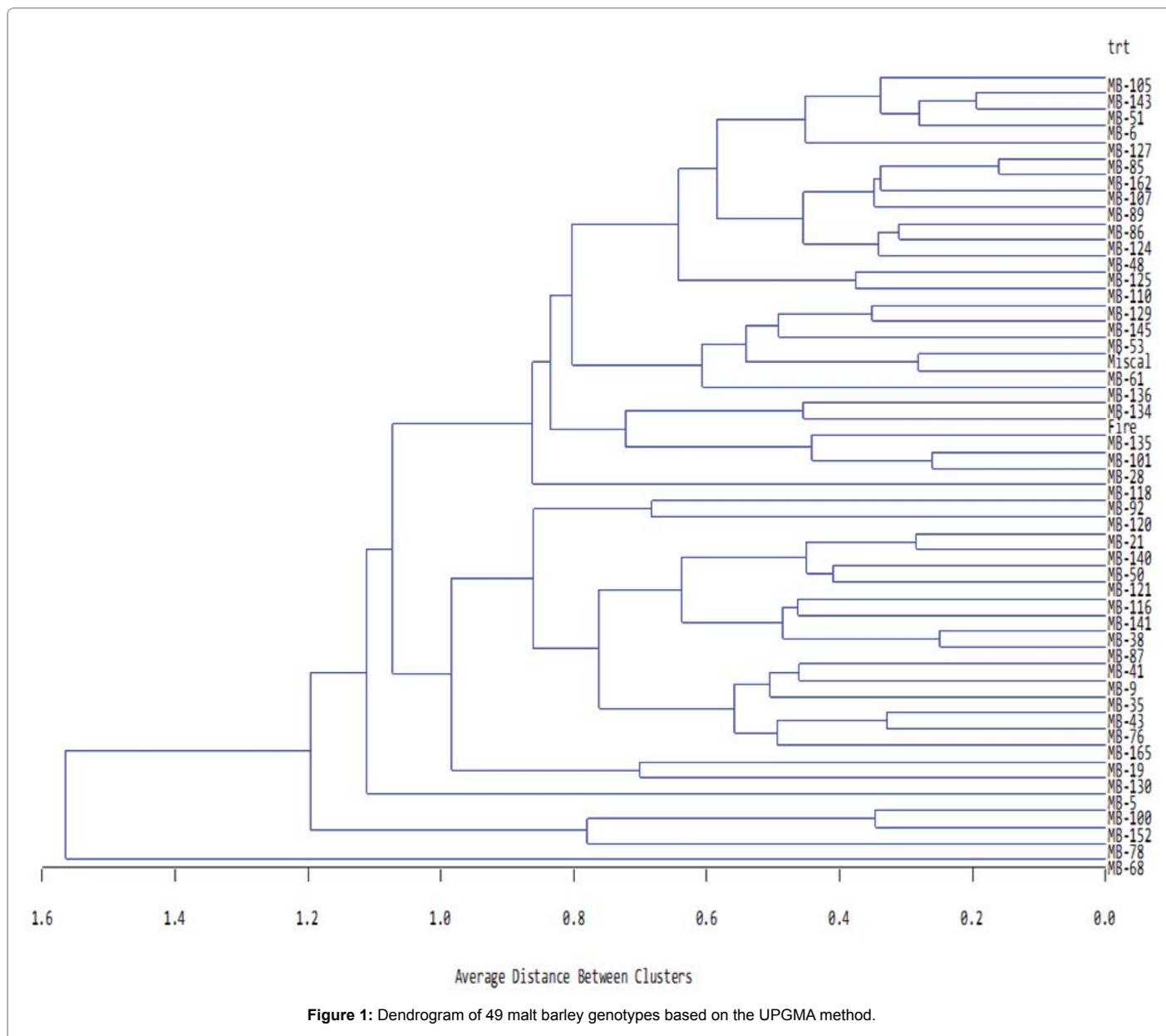


Figure 1: Dendrogram of 49 malt barley genotypes based on the UPGMA method.

Data collection

Data were recorded on each of the five plants randomly selected from the plot of each replication on length, spike length, number of seeds per spike, plant height, number of fertile tiller per plant for all the quantitative and quality characters except for days to heading, days to maturity, thousand seed weight, biomass, protein content, starch content and moisture content in which data were recorded on plot basis from each replication and mean of five plants for each genotypes in each replication was computed for each character and used for statistical analysis.

Data analysis

Genetic divergence analysis: Genetic divergence analysis was computed based on multivariate analysis using Mahalanobis's D^2 statistic (Mahalanobis, 1936) by SAS 9.1 Software program [5,6].

Estimation of squared distances: Squared distances (D^2) for each pair of genotypes combination was computed using the following formula:

$$D_{ij}^2 = (X_i - X_j) S^{-1} (X_i - X_j)$$

Where, D_{ij}^2 = the square distance between any two genotypes i and j ,

X_i and X_j = the vectors for the values for genotype i^{th} and j^{th} genotypes, and

S^{-1} = the inverse of pooled variance covariance matrix.

Principal component analysis

Principal component analysis (PCA) was used to find out the characters, which accounted more to the total variation. The data were standardized to mean zero and variance of one before computing principal component analysis. Principal components based on correlation matrix were calculated using SAS 9.1 computer software [6].

Results

Genetic divergence analysis

Description of genotype collection for agronomical and quality useful characters is important prerequisite for effective and efficient utilization of germplasm collection in breeding program.

Divergence analysis is a technique used to categorize genotypes that are similar into one group and others into different groups. D-square statistics (D^2) developed by Mahalanobis, has been used to classify the divergent genotypes into different groups [5].

Estimation of squared distance (D^2) and clustering of genotypes: The D^2 values based on the pooled mean of genotypes resulted in classifying the 49 genotypes in to nine distinct clusters (Table 1). Clusters I and II were the largest clusters (69.4%) containing thirty four genotypes together. Cluster III and IV (16.3%) had five and three genotypes respectively, Clusters V and VI had 4 genotypes together (8.2%) two genotypes each and Cluster VII, VIII and IX constituted three genotypes (6.1%) with one genotypes each.

Cluster mean analysis: The mean value of the 14 quantitative agronomic and quality characters in each cluster (Table 2).

Cluster II had consisted of fourteen genotypes. This cluster could be characterized by high percentage of moisture content (9.2%), relatively high percentage of starch content (59.78%), relatively short plant height (70.5 cm), late day to maturity (102.3), smallest number of fertile tiller (5.2) next to cluster V and the lowest biomass yield (5.4 tonne per hectare).

Cluster III had consisted of five genotypes including one the standard check Fire-gebis which are characterized by the shortest time to day to heading (64.4) next to cluster V, the highest awn length (12.7 cm) next to cluster IV, the highest plant height (81.5 cm) next to cluster IX and the highest thousand seed weight (47.0 g) among all clusters.

Cluster IV includes three genotypes. The cluster could be characterized by early day to heading (63.8) and day to maturity (95.5) among all clusters, highest thousand seed weight (46.9 g) next to cluster III, the highest percentage of moisture content (9.3%) next to cluster VII, the highest percentage of protein content (17.3%) next to cluster VI, the shortest spike length (6.5 cm) among all clusters, the least number of seed per spike (14.0), the lowest grain yield (0.8 t/ha) among all clusters and the least percentage of harvest index (12.3%) among all clusters.

Cluster V had consisted of two genotypes. The cluster could be characterized by high percentage of starch content (60.6%) next to cluster VII and IX, relatively high percentage of moisture content (9.3%), the highest number of seed per spike (29.9) next to cluster IX, the lowest thousand seed weight (33.2 g), the lowest percentage of protein content (13%) among all clusters, the least number of fertile tiller (5.2) among all clusters, the shortest plant height (68.8 cm) and awn length (9.9 cm) among all clusters.

Cluster VI consisted two genotypes with a characteristic feature of high percentage of protein content (18.5%) among all clusters, the highest biomass yield (8.0 t/ha) next to cluster VII, relatively high percentage of moisture content (9.3%), late day to heading (78 days)

Clusters	Number of genotypes in the cluster	Name of genotypes in the cluster
Cluster I	20	MB-105, MB-85, MB-86, MB-107, MB-51, MB-89, MB-162, MB-48, MB-110, Miscal-21, MB-145, MB-136, MB-124, MB-6, MB-143, MB-53, MB-61, MB-127, MB-129, MB-125
Cluster II	14	MB-50, MB-116, MB-140, MB-35, MB-38, MB-43, MB-76, MB-141, MB-121, MB-87, MB-9, MB-165, MB-21, MB-41
Cluster III	5	MB-134, MB-135, MB-101, MB-28, Fire-gebis
Cluster IV	3	MB-100, MB-78, MB-152
Cluster V	2	MB-120, MB-92
Cluster VI	2	MB-19, MB-130
Cluster VII	1	MB-118
Cluster VIII	1	MB-5
Cluster IX	1	MB-68

Table 1: Distribution of 49 malt barley genotypes in to nine clusters based on D^2 analysis.

Traits	I	II	III	IV	clusters V	VI	VII	VIII	IX
DH	64.1	69.6	64.4	63.8	65.3	78.0	73.0	66.0	69.5
DM	97.7	102.3	96.7	95.5	98.5	106.0	107.0	86.5	102.5
AL	11.8	11.2	12.7	10.5	9.9	10.3	11.1	10.9	12.9
SL	7.9	7.2	7.5	6.5	6.6	7.5	8.2	8.5	6.8
PH	76.8	70.5	81.5	71.5	68.8	69.5	79.9	81.1	93.8
FT	6.1	5.2	7.0	6.5	5.2	5.3	7.9	6.0	7.7
NSPS	21.4	21.3	18.4	14.0	29.9	19.1	22.0	23.9	39.4
GY	2.2	1.3	1.8	0.8	1.3	1.0	2.9	1.4	2.3
BY	6.6	5.4	7.6	6.7	6.0	8.0	9.0	6.0	8.0
HI	33.6	25.7	23.9	12.3	21.9	12.85	32.2	23.3	28.8
TSW	42.1	39.3	47.0	46.8	33.2	42.8	44.8	32.6	38.6
PC	14.6	15.1	15.8	17.3	13.0	18.5	14.4	13.9	14.4
SC	60.4	59.8	59.7	58.5	60.6	57.6	60.1	61.5	60.7
MC	9.0	9.2	8.9	9.3	9.3	9.3	9.4	8.8	8.9

DH: Days to Heading; DM: Days to Maturity; AL: Awn Length; SL: Spike Length; PH: Plant Height (cm); BY: Biomass Yield (t/ha); NFTP: Number of Fertile Tiller per Plant; NSPS: Number of Seed per spike; GY: Grain Yield (t/ha); HI: Harvest Index; TSW: 1000 Seed Weight (g); PC: Protein Content (%); SC: Starch Content (%); MC: Moisture Content (%)

Table 2: Mean value of 14 quantitative agronomic and malt quality characters of the nine clusters for 49 malt barley genotypes.

among all clusters, late day to maturing (106 days) next to cluster VII, the lowest percentage of harvest index (12.8%), the lowest percentage starch content (57.6%) among all clusters. This cluster had intermediate characteristics in other agronomic traits.

Cluster VII had only one genotype. This cluster had features of the highest biomass yield (9.0 t/ha), grain yield (2.9 t/ha), percentage of harvest index (32.2%), number of fertile tiller per plant (7.9) and percentage of moisture content (9.4%) among all clusters, highest spike length (8.2 cm) next to cluster VIII, relatively high awn length (11.1cm), plant height (79.9 cm), thousand seed weight (44.8 g) and percentage of starch content (60.1%), late day to maturity (107 days) among all clusters and relatively late day to heading (73 days).

Cluster VIII had only one genotype. This cluster could be characterized by the highest spike length (8.5 cm) and percentage of starch content (61.5%) among all clusters, high plant height (81.1 cm) next to cluster IX, early day to maturity (86.5) among all clusters and relatively shortest day to heading (66.0), the lowest percentage of moisture content (8.8%) and thousand seed weight (32.6 g) among all clusters and the lowest percentage of protein content (13.9%) next to cluster V.

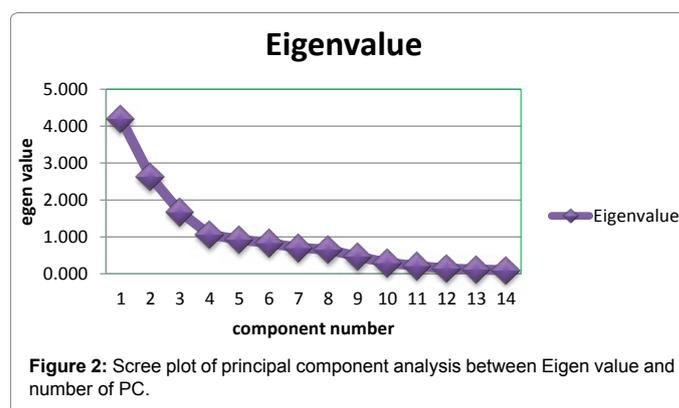
Cluster IX had one genotypes and characterized by its late day to maturity (102.3), low percentage of moisture content (8.9%), smallest spike length (6.8 cm), the highest awn length (12.9 cm), number of seed per spike (39.4) and plant height (93.8 cm) among all clusters, the highest grain yield (2.3 t/ha), biomass yield (8.0 t/ha) and percentage of starch content (60.7%) next to cluster VIII and intermediate characteristics in other agronomic traits.

Principal component analysis: The principal component analysis revealed that the first four principal components, principal component one, principal component two, principal component three and principal component four with eigenvalues 4.2, 2.6, 1.7 and 1.1, respectively accounted 68.3% of the total variation among malt barley genotypes for fourteen quantitative and quality traits (Table 3).

The relative magnitudes of eigenvectors for the first principal component with eigenvalue of about 30% indicated that harvest index

Characters	Eigenvectors			
	PC1	PC2	PC3	PC4
Day to heading	-0.2	-0.3	0.5	0.2
Day to maturity	-0.2	-0.3	0.5	0.2
Awn length	0.3	0.1	0.3	0.5
Spike length	0.3	-0.1	0.1	0.1
Plant height	0.4	0.1	0.3	-0.1
Fertile tiller	0.2	0.3	0.3	-0.1
Number of seed per spike	0.2	-0.3	0.2	-0.4
Grain yield	0.3	0.1	0.0	-0.1
Biomass	0.2	0.3	0.3	-0.5
Harvest index	0.4	-0.1	-0.1	0.3
Thousand seed weight	0.0	0.5	0.0	0.2
Protein content	-0.3	0.4	0.2	0.2
Starch content	0.3	-0.4	-0.2	0.0
Moisture content	-0.4	0.1	0.1	-0.2
Eigenvalue	4.2	2.6	1.7	1.1
Difference	1.6	1.0	0.6	0.1
Percent of total variance explained	30.0	18.8	11.9	7.6
Cumulative percent of total variance explained	30.0	48.7	60.7	68.2

Table 3: Eigen vectors and eigen values of the first four principal components for 14 characters of 49 genotypes of malt barley.



and plant height followed by awn length, spike length, grain yield and starch content were the most important contributing traits. From the second principal component, which contributed 18.8% of the total variation, the most predominant characters thousand seed weight, protein content followed by fertile tiller and biomass Third principal component explained 11.9% of total variation on the characters of day to heading and day to maturity followed by awn length, plant height, fertile tiller and biomass. Finally the fourth principal component contributed 7.6% of the total variation with the traits of awn length followed by harvest index.

Scree plot explains the percentage of variance associated with each principal component obtained by drawing a graph between Eigenvalues and principal component number. The first principal component showed 30% variability with Eigenvalue 4.2 in germplasm, which then reduced gradually (Figure 2).

Discussion

Genetic divergence analysis

Estimation of squared distance (D^2) and clustering of genotypes: Divergence analysis is a technique used to categorize genotypes that are similar into one group and others into different groups. D-square statistics (D^2) developed by Mahalanobis, has been used to classify the divergent genotypes into different groups [5]. Description of genotype collection for agronomical and quality useful characters is important prerequisite for effective and efficient utilization of germplasm collection in breeding program. Diversity among accessions and within accessions showed the potential of genetic variation within accessions which is a source material for barley improving purpose. The exploitation of within accession variation through pure line selection has proven to provide superior germplasm for disease resistance and yield characteristics [9,10].

In this study the result of classifying 49 malt barley genotypes in to nine clusters indicated that the presence of wide diversity or variability among the genotypes (Table 1). The first two clusters accounts 69.3% with thirty four genotypes, therefore these clusters had most genotypes than any other genotypes tested in this study. Clustering of accessions based on the agronomic traits under study revealed no distinct regional grouping patterns in which accessions from same or adjacent regions appeared in different clusters. Same result was reported by Assefa for tef germplasm. Endang et al. [11] stated that type of clustering pattern could be utilized for cross combination to generate the highest possible variability for various important characters.

Quantification of genetic diversity existing within and between groups of genotypes is important and particularly useful in proper

choice of parents for realizing higher heterosis and obtaining useful recombinants. Such clustering pattern of genotypes of different origin is attributed to the free exchange of breeding material from one place to another and or due to unidirectional selection pressure, by the breeders of different locations [12]. Similar results were also reported by Jeena and Arora [13]. Significant differences among varieties for all or majority of the characters would justify further calculation of D^2 [14].

Principal component analysis: Principal component analysis is one of the multivariate statistical techniques which were a powerful tool for investigating and summarizing underlying trends in complex data structures [15]. Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation [12].

Agronomic and malt quality characters having relatively higher value in the first principal component were harvest index, plant height, moisture content, grain yield, starch content and awn length. Characters like thousand seed weight, protein content, number of seed per spike, starch content and fertile tiller had contributed a lot for principal component two; day to maturity, day to heading, biomass yield and plant height had contributed in the third principal component, while awn length, number of seed per spike, biomass yield and harvest index in the fourth principal component were the major contributors to each principal components. The first two principal components, principal component one and two with percentage of total variance values 30% and 48.7%, respectively contributed more to the total variation. Characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero [16]. Therefore, in this study, differentiation of the genotypes into different cluster was because of a cumulative effect of a number of characters rather to the small contribution of each character ($\pm 0.01-0.5$). According to the work of Zaheer et al., the variation studied through Principal Component Analysis revealed that five principal components having greater than 1 eigenvalues contributed 83.40% of the total variation [17]. In this study, four principal components greater than 1 Eigen values contributed 68.3% of the total variation. In addition to the above Scree plot explains the percentage of variance associated with each principal component obtained by drawing a graph between Eigenvalues and principal component number. The first principal component showed 30% variability with Eigen value 4.2 in germplasm, which then reduced gradually (Figure 2). This result showed from the graph obtained, it was concluded that maximum variation was present in first principal component. So selection of genotypes from this principal component will be useful. According to Johnson and Wichern based on the Eigen values and vectors, it is possible to indicate which traits are mainly responsible to explain the variation [18].

In this study among all characters awn length, thousand seed weight, plant height, number of fertile tiller, harvest index, protein content, spike length and grain yield respectively had more contribution to the total diversity of genotypes and they were responsible for the differentiation of the nine clusters. In line with this investigation Abebe Tulu et al. studied the diversity of the Ethiopian barley germplasm through morphological traits and found a considerable diversity for days to heading, days to maturity, biomass and plant height and 1000 grain weight [19]. In another research, Okeno reported significant genotypic variation, for length and width of flag leaf, plant height and yield per plant, indicated possibility of selection response in these traits in spring barley. It may be concluded that the greater divergence in the genotypes due to these characters in the respective clusters would offer a good scope for the improvement of malt barley through rational selection [20].

From this study the principal component analysis confirmed diversity since the entire variation cannot be explained in terms of few principal components. This, in turn indicated the involvement of a number of traits in contributing towards the overall observed diversity. Similarly in highland maize accessions of Ethiopia 71.8% of total variation was accounted by first four principal components [21,22].

Conclusion

Cluster and principal component analysis from quantitative agronomic and malt quality morphological traits indicated the availability of variation within malt barley genotypes to offer a good scope for the improvement of barley through rational selection. This study was conducted for a single environment. Hence, further studies should be conducted to develop suitable genotypes and management to meet the quality requirements of Ethiopian standard and brewing industries.

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Citation: Tilahun A, Alemu F (2017) Traits Diversity Analysis of Malt Barley (*Hordeum vulgare* L.) Genotypes under Irrigation at Koga of West Gojjam in Ethiopia. Mol Biol 6: 190. doi: [10.4172/2168-9547.1000190](https://doi.org/10.4172/2168-9547.1000190)

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