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Genetic Diversity Analysis of Ethiopian Barley (*Hordeum vulgare* L.) Genotypes Based on Agronomic Traits Using Cluster and Principal Component Analysis

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Abstract

Genetic diversity is essential for barley breeding, enabling the selection of desirable traits for improvement. Previous genetic diversity studies on barley have not been sufficient compared to the genetic resources available in the gene bank and ever-changing weather conditions. This study was therefore conducted to assess the extent of genetic variability and association of agronomic traits in barley genotypes at the Adet and Debark experimental stations during the 2023 main cropping season. Eighty-one barley genotypes were evaluated using a 9x9 simple lattice design. The analysis of variance revealed a high level of variability among barley genotypes. The first four principal components at Adet accounted for 78.4% of the total variation, while the first five components accounted for 80.5% at Debark. Traits such as grain-filling period, plant height, biomass, grain yield at Adet, grain yield, grain-filling period, harvest index, and thousand-seed weight at Debark contributed most to the first two components. The biplot analysis also revealed a strong negative correlation between days to heading and leaf rust severity with grain yield, harvest index, and grain filling period. Using hierarchical cluster analysis, the genotypes were grouped into five clusters at each location, each with a specific trait composition. and clusters I and III (71.84). At Adet, the greatest intercluster distance was between clusters II and IV (75.0), followed by clusters II and III (59.35), while at Debark, it was between clusters III and V (103.98), followed by clusters III and IV (82.14). The greater intercluster distance observed in this study indicates genetic diversity among the barley genotypes. This study also identified high-yielding genotypes (5, 9, 18, 32, 41, 50, 51, 53, 54, 60, 65, 68, 75) with grain yield potentials ranging from 2681.2 kg.ha-1 to 7291.6 kg.ha-1 at Debark and 80.5 kg.ha-1 to 4631.8 kg.ha-1 at Adet. These genotypes show potential as varieties for selection or as parents for hybridization. However, multi-year and multi-location trials are needed to confirm these results.

Keywords: biplot analysis, breeding programs, high-yielding genotypes, hybridization, intercluster distance

Introduction

Barley (*Hordeum vulgare*) is a diploid grass species with a chromosome configuration of 2n = 2x = 14 and is a predominantly self-pollinated crop (98%). This high rate of self-pollination contributes to its genetic stability and limits hybridization with species outside its primary gene pool (Dawson et al., 2015; Morrell et al., 2003). Ethiopia, renowned for its extraordinary agro ecological diversity, is a notable center of barley diversity. Over the centuries, this remarkable crop has adapted to a variety of ecological zones within the region, resulting in unique genetic variations found nowhere else in the world (Badr et al., 2000). Notably, the Himalayas, Ethiopia, and Morocco have all, at different points in history, been recognized as vital centers of barley diversity (Bekele, 1983).

Barley's improvement is contingent on genetic diversity. The genetic diversity of barley is crucial for successful barley improvement, and the sources of this diversity can be traced back to a small number of farmer varieties, which are the primary sources of genetic diversity for barley breeding programs (Muñoz-Amatriaín et al., 2014). The choice of parents for breeding is a critical aspect of crop improvement, and separating accessions into morphologically related clusters aids in this process (Abebe et al., 2010).

The genetic diversity of barley is preserved in governmental or international gene banks and germplasm collections at research universities,

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breeding companies, and agricultural organizations across the globe (Parzies et al., 2000). A global inventory of barley genetic resources in ex-situ collections revealed 402,000 accessions across the 47 major barley gene banks, each with more than 500 accessions. (Visioni et al., 2023). The Ethiopian Biodiversity Institute holds a substantial collection of barley germplasm, constituting approximately 20.8% of the total genotypes preserved, with more than 16,000 samples (Ethiopian Biodiversity Institute, 2024).

Genetic diversity is the total genetic variability reflected by differences in DNA sequences, biochemical characteristics, physiological properties, or morphological traits among individuals of a variety or a population. The study of genetic diversity involves analyzing the variation among genotypes using specific methods or a combination thereof (Yogender et al., 2020). Efforts to increase yield depend on assessing and utilizing genetic variability, as crop improvement hinges on the presence of the genetic variability of desirable traits (Swarup et al., 2021).

Barley, a major cereal crop in the Amhara region of Ethiopia, faces significant production challenges due to inefficiency, low productivity of farmer varieties, declining soil fertility, and limited access to improved barley varieties (Lema et al., 2022; Tadesse and Derso, 2019). Overall productivity is adversely affected by such constraints, which affect the livelihoods of smallholder farmers. Despite the various challenges identified in Ethiopia, including insufficient research focused on barley, disease-related issues, and low yield potential in local varieties, the deficiency of superior barley varieties remains a fundamental issue (Weldeyohanis Kifle, 2016). Addressing these issues is crucial for enhancing barley production in the Amhara region.

Several methods are available for diversity analysis, but principal component analysis and clustering analysis were used. In the context of genetic diversity and association studies of agro-morphological traits in barley, principal component analysis has been employed to analyze the relationships among different genotypes based on various characteristics. A study on 48 barley landrace accessions in Ethiopia revealed that the first four principal components contributed significantly to the observed variations, with principal component one alone explaining 49.96% of the total variation. Traits such as grain yield, biomass yield, thousand-grain weight, and plant height substantially contributed to the observed variations in different principal components (Enyew et al., 2019). Research on barley landraces from southern Ethiopia's Gumer district showed significant genetic diversity, with principal component analysis revealing distinct groups among the US, Japanese, and Gumer barley samples (Degu et al., 2023). Another study on a large collection of barley germplasms in India demonstrated that principal component analysis (PCA) helped identify key traits contributing to variance, such as plant height, grains per spike, spike length, grain yield, and days to spike emergence and maturity (Kaur et al., 2022).

Genetic divergence and cluster analysis are fundamental concepts in population genetics and evolutionary biology. These methods are particularly relevant in studying agronomic traits in barley genotypes. Genetic divergence refers to the process by which two or more populations of an organism accumulate independent genetic changes over time, often leading to the formation of new species (De Ron and Rodiño, 2023). Cluster analysis, on the other hand, is a statistical method used to group individuals based on their genetic similarities (Bekis et al., 2021). It is often used in population genetics to identify distinct genetic clusters or populations within a species. It is a statistical technique. Researchers can identify distinct genetic groups and their interconnectedness by clustering these genotypes based on their genetic markers or traits. This information is valuable for breeding programs and conservation initiatives (Farooqi et al., 2023). When genotypes are clustered, breeders can select parents from different clusters, facilitating the creation of hybrid genotypes that combine desirable traits from multiple sources (Alqudah et al., 2020).

This study, with significant implications for academics and society, provides insight into heritable features and a way to develop successful breeding programs. The objective of this study was to assess the extent of genetic diversity, explore the associations among agronomic traits within barley genotypes, classify barley genotypes based on genetic similarity and performance characteristics, identify unique clusters, identify key traits contributing to genetic variability, and ultimately identify the most promising barley genotypes based on their performance.

Materials and Methods

Description of the Study Area

The experiment was conducted at the Adet and Debark experimental stations of the Amhara Regional Agricultural Research Institute, Ethiopia, during the 2023 main cropping season under rain-fed growing conditions (Table 1, Figure 1).

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Table 1. Description of the study areas in Adet and Debark, Ethiopia

Location	Altitude (m.a.s.l)	Latitude	Longitude	Soil type	Average rainfall	Temperature ranges
Adet	2240	17°16′45.4″N	37°29′16.8″E	Nitisol	1100.2	8.6-19.8°C
Debark	2885	13°13′16.6″N	37°89′91.2″E	Cambisol	974	28.4-12.6°C

Notes: m a.s.l. = meters above sea level. The weather variables were sourced from the National Meteorological Agency of Ethiopia (Abunu et al., 2024; Birhanu et al., 2020).

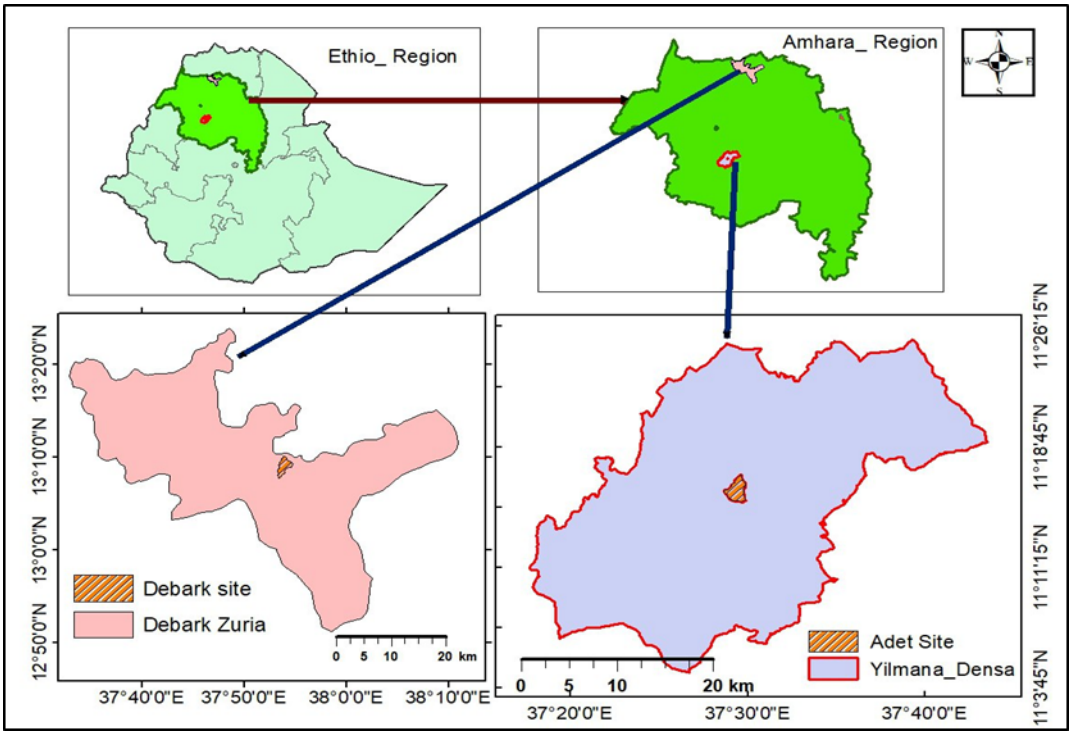

Figure 1. A map shows the study area's location, Adet and Debark sites.

Genetic Materials

The experiment was conducted using one local check, two standard checks (Hagerie and HB-1307), and 78 genotypes of barley for Adet and three standard checks (Debark-1, Hagerie and HB-1307) with 78 similar genotypes of barley. The Gonder Agricultural Research Centre kindly donated the genotypes, where purification was performed over three years using the single-seed selection method. These genotypes initially originated from the Institute of Biodiversity of Ethiopia (25 genotypes) from the Holetta Agricultural Research Center (47 genotypes), and the remaining six genotypes were collected at the Gonder Agricultural Research Centre. All the genotypes used in this study are listed in Tables 2 and 3.

Field Experiment

The experiment was laid out according to a 9 × 9 simple lattice design with two replications in six rows (2 m width), 2.5 m in length, and 0.2 m in-row spacing, with four harvestable rows. The spacing between plots was 0.4 m, and between blocks was 1 m. The average seed rate used was 125 kg.ha-1 or 37.5 g per plot, and the plants were drilled manually. The plots received fertilizer as per the recommendation, 121 kg.ha-1 of NPS and 200 kg.ha-1 of urea. One-third of the urea was applied at planting, and two-thirds of the urea was applied at tillering. All NPS was applied during planting. The plots were hand-weeded during the critical period for barley, which spans from the early tillering stage, when secondary shoots emerge from the base of the plant to the jointing stage, when the barley stem begins to elongate, and the first node becomes visible above the soil surface.

Data Collection

Data were collected based on the International Plant Genetic Resources Institute barley descriptor (IPGRI, 1994). Grain yield and thousand seed weight were adjusted for 12.5% moisture content. Data were

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Table 2. List of barley genotypes with entry codes, source organization, and unique Genotype codes or pedigrees

Entry code	Source organization*	Pedigree	Genotype unique code
5	HARC	HB42/HB1966	HBFB19-0114
6	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0780
7	HARC	(IBON 93/91 x EH 1493)/HB1307	HBFB19-0389
9	HARC	(HB 42 x Su-lilly)/(EH 1493 x HB 1307)	HBFB19-0210
14	HARC	Gobae/IBON 13/2	HBFB18-0457
15	HARC	Gobae/IBON 13/2	HBFB18-0431
16	HARC	HB 1966 x SK 24	HBFB19-1279
17	HARC	HB42/HB1966	HBFB19-0101
18	HARC	HB1307/HB42	HBFB18-0095
23	HARC	Acc.# 17148/IBON 13/33	HBFB18-0811
24	HARC	Shege/HB1964	HBFB19-0022
25	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0767
26	HARC	(IBON 93/91 x Cross 41/98)/(MB F4 2015 P# 1)	HBFB19-0804
27	HARC	(IBON 93/91 x Cross 41/98)/HB 1966	HBFB19-1007
32	HARC	IBON-HI 134/20	HBFBN20-2541
33	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0790
34	HARC	(IBON-HI 13/14 P# 49) x HB 1964	HBFB19-1215
35	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0779
36	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0767
41	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0769
42	HARC	M-21/IBON 13/14-49	HBFB18-1777
43	HARC	M 135/HB 1307	HBFB18-0602
44	HARC	HB1307/HB42	HBFB18-0094
45	HARC	HB42/HB1966	HBFB19-0122
49	HARC	(IBON 93/91 x Cross 41/98)/(MB F4 2015 P# 1)	HBFB19-0829
50	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0744
51	HARC	(IBON 93/91 x Cross 41/98)/(MB F4 2015 P# 1)	HBFB19-0799
52	HARC	IBON 14/15-96/IBON 13/33	HBFB18-0108
53	HARC	Acc.# 17148/IBON 13/33	HBFB18-0829
54	HARC	Acc.# 17148/IBON 13/33	HBFB18-0827
55	HARC	(IBON-HI 13/14 P# 49) x HB 1964	HBFB19-1221
56	HARC	Gobae/IBON 13/2	HBFB18-0452
58	HARC	(Estayesh x HB 1307) x HB 1964	HBFB19-1149
59	HARC	M 135/HB 1307	HBFB18-0598
60	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0766
61	HARC	(IBON-HI 13/14 P# 49) x HB 1964	HBFB19-1233
64	HARC	(IBON 93/91 x EH 1493)/(MB F4 2015 P# 1)	HBFB19-0269
65	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0765
66	HARC	(IBON 93/91 x Cross 41/98)/(Cross# 41/98 x HB 42)	HBFB19-0917
67	HARC	(HB 1307 X Estayesh)/HB 1964	HBFB19-0761
68	HARC	(HB 42 x Su-lilly)/(EH 1493 x HB 1307)	HBFB19-0181
69	HARC	HB42/HB1966	HBFB19-0098
72	HARC	(HB 42 x Su-lilly)/HB 1966	HBFB19-0255
73	HARC	MN Brite/IBON 13/2	HBFB18-2470
74	HARC	Acc.# 17148/IBON 13/33	HBFB18-0832
75	HARC	M-21/IBON 13/14-49	HBFB18-1751
76	HARC	(HB 42 x Su-lilly)/(EH 1493 x HB 1307)	HBFB19-0185

Notes: *HARC = Holetta Agricultural Research Center

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Table 3. List barley genotypes with entry codes, source organizations, and accession numbers.

Entry code	Source organization	Accession number	Entry code	Source organization	Accession number
62	EBI	3254	63	EBI	3290
1	EBI	1832	70	EBI	3282
10	EBI	2161	71	EBI	3726
11	EBI	1654	77	EBI	1697
12	EBI	1662	78	EBI	3238
19	EBI	1614	79	EBI	4112
20	EBI	1777	80	EBI	6216
22	EBI	1613	81	EBI	5568
28	EBI	1739	2	GARC	17
30	EBI	3322	3	GARC	26
31	EBI	3315	4	GARC	5
37	EBI	3703	13	GARC	111
38	EBI	1653	21	GARC	23
39	EBI	3655	47	GARC	92
40	EBI	1719	8	GARC	Debark-1/Local
46	EBI	3658	29	Holeta ARC	HB1307
48	EBI	3302	57	DBARC	Hagere

Notes: EBI = Ethiopian Biodiversity Institute; GARC = Gonder Agricultural Research Center; DBARC = Debre Brihan Agricultural Research Center

collected on fourteen quantitative traits, including plant height, awn length, spike length, culm length, and number of seeds per spike, and measured across ten randomly selected plants. Photos depicted barley growth in the field are in Figure 2. Traits such as days to heading, days to maturity, the grain filling period, grain yield, thousand seed weight, the harvest index, and aboveground biomass were measured and recorded on a plot basis. Harvest index was estimated by dividing the average grain yield by the average biological yield (Pennington, 2013):

$$\text{Harvest Index (\%)} = \frac{\text{grain yield (kg)}}{\text{Biological yield (kg)}} \times 100$$

Disease assessments for leaf rust (*Puccinia hordei*) and net blotch (*Pyrenophora teres* f. teres) severity were conducted during the grain-filling period via established scales. Leaf rust severity was assessed at the peak stage of the disease during the grain-filling period using the modified Cobb's scale, a recognized method described by (Peterson et al., 1948), which quantifies disease severity on a scale of 0 to 100% (Table 2).

For net blotch, disease assessment was carried out at the peak stage of the disease during the grain-filling period following the pictorial guide, which provides a standardized scale for measuring disease severity. This allows for accurate and consistent recording of the impact of net blotch, spot blotch, and scald severity on the barley plants involved in the experiment, as outlined in Table 2 (Manandhar et al., 2016).

Data Analysis

The collected data were subjected to statistical analysis according to the simple lattice design procedure. SAS computer software version 9.4 and R software version 4.3.2 were used for the statistical analysis.

Analysis of variance

The relevant data generated on a plot and plant basis were checked for assumptions of analysis of variance (ANOVA), and traits that violated the assumption of ANOVA (i.e., leaf rust severity) were transformed by square root transformation. The data were then subjected to analysis of variance (ANOVA) following Gomez and Gomez 1984 and the restricted (or residual, or reduced) maximum likelihood (REML) approach, which can produce unbiased estimates of variance and covariance parameters (Rameez et al., 2022) using R software version 4.3.2. The results obtained from the homogeneity test for error of variance rejected the hypothesis of homogeneous variance; therefore, the analysis of variance and other statistical analyses were performed separately for the two locations.

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Table 4. Assessment for disease-infected leaves

Scale	Leaf area covered by disease (%)
1	< 5
3	5-10
5	1-25
7	26-50
9	> 50

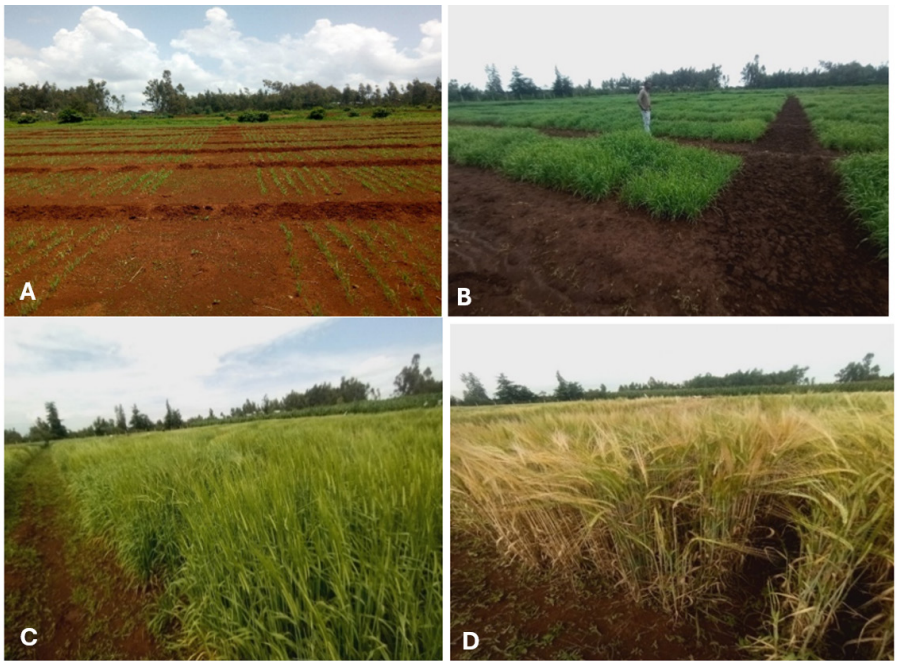

Figure 2. Partial field observation in the study area at Adet, Ethiopia, at 15 (A), 40 (B), 70 (C), and 90 days (D) after planting

Principal component analysis

Principal component analysis was performed using the latest version of R software after standardizing the variables because they have different units of measurement, which prohibits each variable from receiving an equal weight in the analysis. The principal component based on the correlation matrix was calculated using R statistical software version 4.3.2, the facto extra R package (Irnawati et al., 2020).

Genetic divergence and clustering of genotypes

The hierarchical cluster analysis technique was utilized to group barley genotypes based on their genetic profiles or 14 agro morphological traits studied at two distinct locations (Table 1). Cluster analysis assembled those barley genotypes into five distinct clusters, with varying compositions based on the specific location. Clustering was performed after standardizing the data using R software's latest version of the clustering procedure. To determine the optimum number of clusters, the silhouette method, which is a graphical aid to the interpretation and validation of cluster analysis in R, was utilized (Rousseeuw, 1987).

Genetic divergence analysis was performed based on multivariate analysis using Mahalanobis's D2 statistic by R software's version R 4.3.2 (Mahalanobis, 2018). Average intra-cluster $D^2 = \Sigma Di^2/n$ where, ΣDi^2 = sum of all distances between all possible combinations (n) of the genotypes included in the cluster and average inter-cluster distance $D^2 = \Sigma Dij^2/ni\ldots. nj$ where, ΣDij^2 = sum of all distances between all possible combinations (ni. nj) of the genotypes between the clusters (ni = number of genotypes in i^{th} cluster, nj = number of genotypes in j^{th} cluster) was calculated using SAS version 9.4.

The significance of the squared distances for each cluster was tested against the tabulated $\chi 2$ (chi-square) values at 1% and 5% probability levels, where p = number of traits used for clustering genotypes using SAS version 9.4. The contribution of individual character to the total divergence and cluster means

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of barley genotypes falling under different clusters was also calculated by the method employed (Singh and Chaudhary, 1977).

Results and Discussion

Analysis of Variance

The homogeneity test for error of variance for grain yield was <0.001, which suggested enough evidence to conclude that the variances at each location were significantly different. Therefore, variance and other statistical analyses were performed separately for the two locations. An analysis of variance revealed highly significant ($p<0.01$) differences in all evaluated traits at both locations, as shown in Tables 5 and 6. This finding indicates high genetic diversity among the genotypes, revealing numerous options for selecting desired traits and creating opportunities to improve barley genotypes. These findings emphasize the significant trait variations, forming a solid foundation for targeted breeding programs to improve specific barley traits.

The study aligns with previous findings by Hailu et al. (2016a), who reported significant genetic variability in all traits except for 1000-seeds weight at Quiha ($p<0.05$) and non-significant plant height at Atsbi and Ofla. Similarly, a study by Ayichew (2019), indicated a highly significant ($p<0.01$) difference among barley genotypes for most of the traits except awn length. Shiferaw et al. (2020) also revealed a significant difference in all traits except days to emergence using 320 Ethiopian barley genotypes. In addition, other studies on barley confirmed a significant phenotypic diversity and variation in the highlands of Bale and Southern Ethiopia (Angassa and Mohammed, 2021; Gadissa et al., 2021; Gadissa and Gudeta, 2023).

Principal Component Analysis

Principal component analysis of 14 quantitative traits from Adet revealed that the first four principal components with eigenvalues greater than one accounted for 78.4% of the total variation among the genotypes. The first principal component explains 37.9% of the total variance, with an eigenvalue of 5.3. This finding suggested that the first principal component captures a substantial portion of the information in the data. The second principal component explained an additional 16.5% of the variance, bringing the cumulative variance to 54.4% (Table 7). The first seven dimensions explain more than 93% of the total variance in the data. This indicates that the first seven dimensions' capture most of the variability in the data, while the remaining dimensions contribute less significantly. Similarly, a study by Fantahun et al. (2023), on another set of 320 barley genotypes reported that the first three principal components, each with an eigenvalue greater than one, explained about 70% of the total variation concerning ten measured traits. In a study conducted by Allo Aman et al. (2020), an analysis of thirteen quantitative traits revealed that the first three principal components (PCs), each with an Eigenvalue >1, account for 51.75% of the total variation among the genotypes tested. The first principal component accounts for 22.56% of the total variance, and the second and third principal components account for 18.95 and 10.25% of the total variation, respectively. This study aligns with the findings of Enyew et al. (2019), who performed principal component analysis on 52 barley genotypes for 12 traits, and the first four principal components were identified as having the most significant contributions to the observed variations. These principal components accounted for 80.79% of the total variation for the 12 traits under study. This demonstrates how principal component analysis can enable breeders to focus on specific traits of interest for crop improvement.

The first five principal components with eigenvalues greater than one accounted for 80.5% of the variation across the 14 traits under study at Debark and had a considerable impact on the observed variations (Table 7). The first principal component explains 25% of the variance, with an eigenvalue of 3.5. This is less than the variance explained by the first principal component in Adet, suggesting that the data in Debark is more spread out across the dimensions, as Ghonaim et al. (2023) stated. The second principal component explains an additional 22.3% of the variance, bringing the cumulative variance to 46.7%. The first eight dimensions explained over 92.8% of the total variance in the data. A similar study by Hailu et al. (2016b), was conducted in Atsbi, Ofla, and Quiha in the Tigray region. Principal component analysis revealed that in the Atsbi environment, principal components one to four, with eigenvalues 3.87, 2.87, 1.26, and 1.04, accounted for 35.22%, 26.09%, 11.42%, and 9.43% of the variation respectively, totaling 82.16%. In Ofla, the first three principal components, with eigenvalues 4.08, 2.07, and 1.47, explained 37.07%, 18.80%, and 13.40% of the variation respectively, totaling 69.27%. In China, the first three components, with eigenvalues 4.64, 1.93, and 1.35, accounted for 42.20%, 17.58%, and 12.26% of the variation, respectively, totaling 72.04%. In the study conducted by Jalata et al. (2020), the variation among barley genotypes was examined using principal component analysis. The analysis identified four principal components that accounted for a significant portion of the total variation. Specifically, the first principal component accounted for 32.7%

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Table 5. Analysis of variance (ANOVA) for 14 quantitative traits of 81 barley genotypes tested at Adet during 2023

Traits	Mean square				CV%	R^2	Mean	RE over RCBD
	Genotype (80)	Error (64)	Rep (1)	Block (16)				
Days to heading	83.8**	3.41	18.67	11.02	3.0	0.963	66.2	127.0
Days to maturity	30.01**	2.58	3.27	4.62	1.5	0.942	102.5	106.5
GFP	49.9**	3.49	6.32	6.66	5.7	0.933	36.2	107.9
Plant height	252.19**	25.66	0.06	34.75	4.5	0.937	98.7	104.6
Culm length	236.65**	25.15	0.89	33.59	4.8	0.934	91.5	104.2
Awn length	3.86**	0.88	7.51	2.15	8.0	0.837	12.4	115.2
Spike length	2.09**	0.33	1.43	0.34	7.8	0.886	7.2	100.0
SPS	266.55**	12.19	46.29	13.29	8.6	0.962	40.5	100.2
1000-seed weight	112.11**	7.25	13.00	5.67	9.4	0.946	28.7	95.2
Above ground biomass	8.19**	0.87	0.43	1.83	11.3	0.919	8.7	110.6
Grain yield	2221624**	228814	1051720	289098	20.4	0.928	2386.8	101.1
Harvest index	154.37**	20.75	84.06	30.94	18.2	0.892	26.1	103.0
Rust severity	11.21**	1.48	7.11	3.33	30.3	0.960	4.1	112.5
Net blotch	0.36**	0.07	0.95	0.15	15.8	0.870	1.7	109.6

Notes ** = highly significant according to ANOVA

Table 6. Analysis of variance (ANOVA) for 14 quantitative traits of 81 barley genotypes tested at Debark during 2023

Traits	Mean square				CV%	R^2	Mean	RE over RCBD
	Genotype (80)	Error (64)	Rep (1)	Block (16)				
Days to heading	29.36**	2.71	16.69	6.30	2.4	0.920	76.5	113.6
Days to maturity	8.07**	2.27	0.61	5.18	1.3	0.794	125.8	113.1
Grain filling	19.47**	3.19	23.73	4.10	3.8	0.872	49.4	101.2
Plant height	101.52**	11.90	24.04	44.44	3.1	0.916	112.4	135.0
Culm length	87.17**	10.63	25.68	47.60	3.4	0.911	104.9	146.9
Awn length	2.62**	0.91	6.16	0.74	7.5	0.777	12.66	95.6
Spike length	1.0885**	0.28	2.25	0.38	7.2	0.836	7.3	101.9
No. seed/spike	110.16**	12.01	13.12	12.09	6.5	0.922	50.9	101.3
1000-seed weight	119.3**	9.35	4.77	13.96	8.0	0.933	39.77	101.3
Dry biomass	5.387**	2.11	2.90	7.97	8.2	0.817	17.74	135.6
Grain yield	2167836**	646915	1462145	927549	13.8	0.826	5664.8	102.5
Harvest index	52.189**	18.07	10.89	15.58	21.0	0.768	31.9	100.5
Leaf rust severity	8.352**	1.59	11.13	1.86	33.8	0.863	3.7	100.5
Net blotch severity	0.339**	0.13	11.1	1.86	22.2	0.767	1.6	100.8

Notes: ** = highly significant according to ANOVA

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Table 7. Eigenvalues, standard deviation, and percentage of total variance explained by each principal component at Adet and Debark, Ethiopia

	Adet				Debark				
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC5
Eigenvalue	5.3	2.3	2.1	1.3	3.5	3.1	1.9	1.7	1.1
Variance percent	37.9	16.5	14.8	9.1	24.7	22.3	13.2	12.3	7.9
CV%	37.9	54.4	69.2	78.4	24.7	47.0	60.3	72.6	80.5
SD	2.3	1.5	1.4	1.1	1.9	1.8	1.4	1.3	1.0

Notes: PC= principal component; CV% = cumulative variance percent; SD = standard deviation

of the variation, the second for 22.4%, the third for 16.7%, and the fourth for 11.6%. Collectively, these four components explained approximately 83.4% of the total variation.

Contribution of Traits toward the First Two Principal Components

The pattern matrix Eigenvectors resulting from principal component analysis, as presented in Table 8, provide Eigenvectors (loadings) of measured traits on the extracted components for the barley genotypes assessed at Adet. A loading represents the correlation between the original variable and the component, thus indicating the contribution of that trait to the component. The traits with the highest absolute loadings on each principal component are the ones that contribute most to the variation explained by that principal component.

In Adet, the first principal component is positively influenced by grain filling period (0.36), plant height (0.26), culm length (0.26), spike length (0.21), harvest index (0.31), thousand seed weight (0.31), aboveground biomass (0.39) and grain yield (0.37). On the other hand, days to heading (-0.3) and leaf rust severity (-0.3) influence principal component one, indicating an inverse relationship with the traits. The second principal component in Adet is negatively influenced by days to maturity (-0.55), days to heading (-0.41), awn length (-0.32), net blotch severity (-0.34), thousand seed weight (-0.26), and spike length (-0.25), suggesting these traits contribute to this variability in the opposite direction. The number of seeds per spike (0.29) and leaf rust severity (0.25) positively influence principal component two, indicating a different dimension of variability. In the case of the study at Adet, certain agronomic traits were found to have relatively higher value in the first two principal components grain yield, grain filling period, aboveground biomass, and days to heading. Traits like thousand seed weight, days to maturity, harvest index, and leaf rust disease had contributed moderately to the first two principal components. In contrast, spike length, awn length, number of seeds per plant, and severity to net blotch disease were the least contributors to the first two principal components. This suggests that these traits had a minimal influence on the variability of the data. In another study by Angassa and Mohammed (2022), the principal component analysis results indicated that the first two principal components with eigenvalues more than 1 (1.74 and 4.30) contained variability of 21.80% and 53.77%, respectively. In the principal component, the number of seeds per spike (0.91), thousand seed weight (-0.87), and spike length (-0.85) contributed to high variability compared to the rest of the traits. The principal component two was mainly influenced by grain yield (0.83) and plant height (0.69) with positive loading.

The pattern matrix resulting from principal component analysis, as presented in Table 9, provides eigenvectors (loadings) of measured traits on the extracted components for the barley genotypes assessed at Debark. In Debark, the first principal component is positively influenced by grain filling (0.41), thousand seed weight (0.14), aboveground biomass (0.2), grain yield (0.443), and harvest index (0.44), like Adet. However, days to heading (-0.4) and leaf rust severity (-0.31) negatively influence principal component one, contrasting on days to heading with the pattern observed in Adet. The second principal component in Debark is positively influenced by days to maturity (0.23), plant height (0.36), culm length (0.34), awn length (0.29), 1000-seed weight (0.44), and above dry ground biomass (0.31). Leaf rust severity (-0.23) and number of seeds per spike (-0.29) negatively influence principal component two, indicating a different dimension of variability. In a study at Debark, agronomic traits had relatively higher values in the first two principal components: grain yield, grain filling period, harvest index, and thousand seed weight. Traits like days to heading, plant height, and leaf rust disease had contributed moderately to the first two principal components. In contrast, aboveground biomass, spike length, awn length, number of seeds per plant, days to maturity, and net blotch severity were the least contributors to the first two principal components. In a study by

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Table 8. Eigenvalues, Eigenvectors, and percentage of total variance explained by each principal at Adet.

Traits	Adet			
	PC1	PC2	PC3	PC4
Days to heading	-0.3	-0.41	-0.01	0.2
Days to maturity	-0.04	-0.55	0.15	0.21
Grain filling	0.36	0.09	0.13	-0.1
Plant height	0.26	-0.08	-0.51	0.03
Culm length	0.25	-0.06	-0.52	0.07
Awn length	0.05	-0.32	-0.33	0.26
Spike length	0.21	-0.25	-0.1	-0.43
No. seed/spike	0.13	0.29	-0.13	0.6
1000-seed weight	0.31	-0.26	-0.03	-0.4
Dry biomass	0.39	-0.02	0	0.15
Grain yield	0.37	0.09	0.24	0.09
Harvest index	0.31	0.12	0.31	0.1
Leaf rust severity	-0.30	0.25	-0.23	-0.26
Net blotch severity	0.07	-0.34	0.3	0.12

Allo Aman et al. (2020), the first principal component was heavily influenced by traits such as plant height, thousand seed weight, grain yield, and peduncle length in a positive direction, while days to 50% heading had a strong negative loading. The second principal component saw major contributions from the number of seeds per spike, days to 90% maturity, and spike length.

In both cases, the first few components explain much of the variance in the data, allowing us to effectively reduce the dimensionality of our data without losing too much information. This information can be used to understand which traits are most important in explaining the genetic diversity of barley genotypes in these two locations. The remaining components explain less and less variance, which can often be ignored without significantly impacting the analysis. However, the decision on how many components to retain should also consider the research question and the complexity of the dataset.

Genotype by Trait (GT) Biplot Analysis and Correlation Among traits

Line-by-trait biplot analysis is a statistical method used in genetics and breeding. This method is beneficial for visualizing and interpreting multivariate data, such as the data obtained from evaluating multiple traits across different genotypes or lines. According to Faheem et al. (2023), in a line-by-trait biplot, each line (or genotype) is represented as a point, and each trait is described as a vector. The origin of the biplot represents the average values for all traits investigated.

For the case of Adet, genotypes situated in the bottom left quadrant of the biplot were closely associated with traits such as high heading and maturity dates. Genotypes in the top left quadrant were primarily associated with high severity of leaf rust disease. Those in the top right quadrant were linked with high grain yield, harvest index, grain filling period, and number of seeds per plant. Genotypes in the bottom right quadrant were characterized by high biomass, thousand seed weight, long spike length, plant height, and culm length. Genotypes near the origin of the biplot shared similar genetic characteristics and indicated less divergence for the studied 14 traits, while those far from the origin were considered genetically distinct. The distribution of genotypes across the four quadrants indicates significant genetic diversity among the barley genotypes studied at Adet (Figure 3 A).

In the case of Debark, the genotypes in the top left quadrant were closely associated with late heading, maturity, extended plant height, and culm length. Genotypes in the bottom left quadrant were primarily associated with high leaf rust severity. Those in the bottom right quadrant had a high harvest index and number of seeds per spike. The genotypes in the top right quadrant were characterized by high grain yield, aboveground biomass, a long grain filling period, and high thousand seed weight. Like Adet, genotypes near the origin of the biplot shared similar genetic characteristics, while those far from the origin were considered genetically distinct. The distribution of

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Table 9. Eigenvalues, eigenvectors, and percentage of total variance explained by each principal component at Debark

Traits	PC1	PC2	PC3	PC4	PC5
Days to heading	-0.40	0.03	-0.46	-0.03	0.15
Days to maturity	-0.16	0.23	-0.51	0.01	-0.33
Grain filling	0.41	0.11	0.25	0.05	-0.41
Plant height	-0.20	0.36	0.13	0.46	0.12
Culm length	-0.20	0.34	0.13	0.49	0.07
Awn length	-0.02	0.29	0.16	0.02	0.06
Spike length	-0.06	0.37	0.05	-0.19	0.51
No. seed/spike	0.11	-0.29	-0.25	0.46	0.12
1000-seed weight	0.14	0.44	0.22	-0.17	-0.10
Dry biomass	0.20	0.31	-0.35	0.11	-0.38
Grain yield	0.45	0.11	-0.29	0.10	0.15
Harvest index	0.43	-0.05	-0.13	0.06	0.44
Leaf rust severity	-0.31	-0.23	0.23	0.09	-0.18
Net blotch severity	-0.11	0.16	-0.15	-0.48	0.03

Notes: *HARC = Holetta Agricultural Research Center

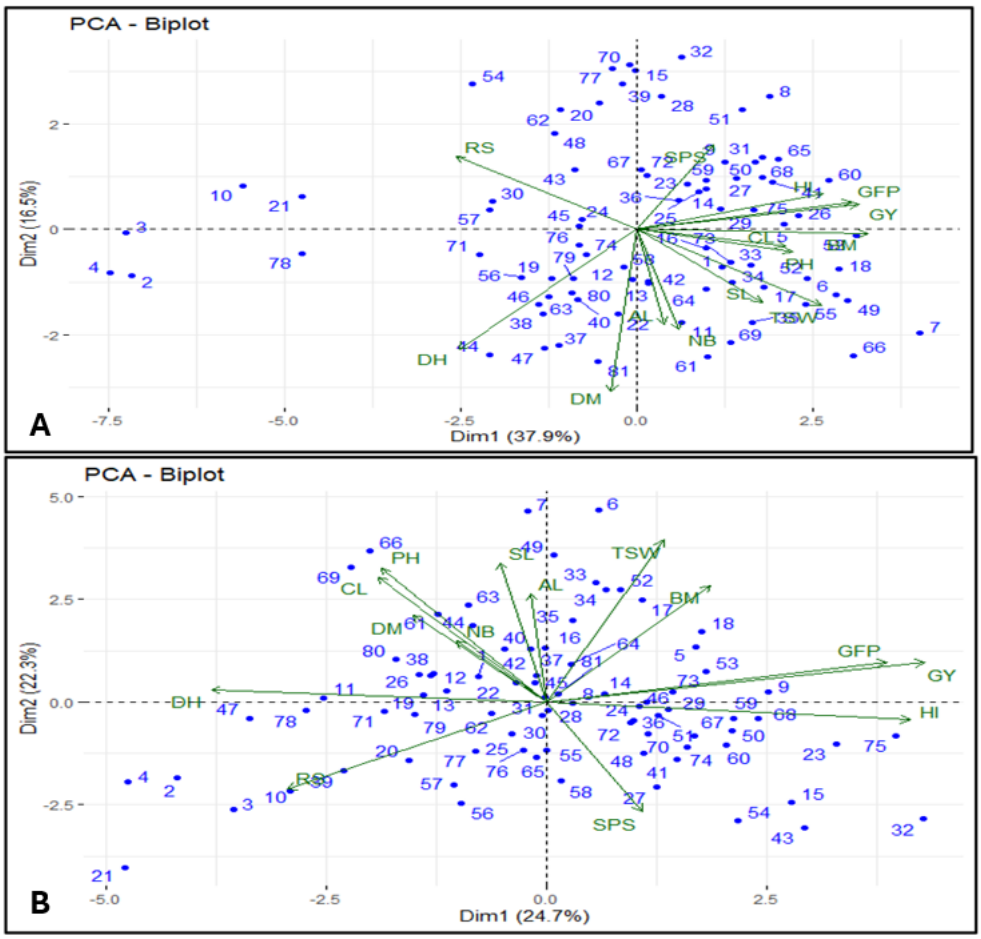

Figure 3. Biplot (axes PC1 and PC2) of 14 quantitative traits of 81 barley genotypes at Adet (A) and Debark (B), Ethiopia

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genotypes across the four quadrants also indicated significant genetic diversity among the barley genotypes studied at Debark (Figure 3B).

The angle between any two vectors (traits) provides information about the correlation between those traits. An angle of 90° indicates zero correlation, or less indicates a positive correlation, whereas an angle greater than 90° signifies a negative correlation. This type of bi-plot analysis can reveal how different traits and genotypes are associated with each other. For instance, it can show which genotypes have high values for traits and low values for others. This information can be valuable for breeders, as it can guide the selection of parent lines for breeding programs (Faheem et al., 2023).

A biplot was generated for the first two principal components against each other to determine associations and genetic diversity among the currently studied barley genotypes and traits (Figure 3. A and B). The results of the present study indicated a wide distribution of genotypes on the biplot, which indicated the presence of high genetic diversity. The biplot analysis conducted in this study also provided a clear visualization of the relationships among various agronomic traits. One of the key findings was the strong negative correlation between the days to heading and leaf rust severity and grain yield, harvest index, and grain filling period. This was observed in the dimensional space defined by the first two principal components at both locations. This finding suggested that genotypes that head later and have greater leaf rust severity tend to have lower grain yields, harvest indices, and grain filling periods.

On the other hand, several traits positively correlated at both locations. These included grain yield, harvest index, grain filling period, aboveground biomass, thousand seed weight, spike length, and plant height. This indicates that genotypes with higher values for one of these traits tend to also have higher values for the others. For instance, genotypes with higher grain yield also tend to have a more extended grain filling period, greater aboveground biomass, heavier thousand seed weight, longer spikes, and taller plants. These correlations among traits can be leveraged in breeding programs to select desirable combinations of traits. A similar finding was reported by Gadissa et al. (2021); the principal component analysis loading plot showed a very strong and close correlation among such traits as grain yield, number of seeds per spike, and thousand seed weight. Moreover, a strong positive correlation exists among the remaining traits except plant height.

Clustering and Genetic Divergence Analysis

Hierarchical clustering

The hierarchical cluster analysis technique was utilized to group barley genotypes based on their genetic profiles or 14 agro morphological traits studied at two distinct locations. Cluster analysis assembled those barley genotypes into five distinct clusters, varying compositions based on the specific location. The means for the agro morphological traits of each cluster of barley genotypes were tested at Adet and Debark and are presented in Table 10 and Table 11.

Table 10. Cluster membership profile of 81 barley genotypes based on 14 traits at Adet, Ethiopia

Cluster	Number of genotypes	Percentage (%)	Entries code
I	27	33.3	1, 11, 12, 13, 16, 17, 19, 22, 24, 37, 38, 40, 42, 44, 45, 46, 47, 52, 56, 63, 64, 69, 74, 76, 79, 80, 81
II	4	4.9	2, 3, 4, 21
III	29	35.8	5, 9, 14, 15, 18, 23, 25, 26, 27, 29, 31, 32, 36, 41, 43, 48, 50, 51, 53, 54, 58, 59, 60, 65, 67, 68, 72, 73, 75
IV	10	12.3	6, 7, 8, 33, 34, 35, 49, 55, 61, 66
V	11	13.6	10, 20, 28, 30, 39, 57, 62, 70, 71, 77, 78

As detailed in Table 7, Cluster I at Adet comprised 27 genotypes representing 33.3% of the total experimental materials. Late-maturing plants are characterized by a moderately extended plant height, thousand seed weight, number of seeds per plant, aboveground biomass, moderate susceptibility to leaf rust disease, and low-yielding genotypes. Cluster II, the smallest cluster, comprised only four genotypes, representing 4.9% of the total at Adet. Cluster II had the highest mean values for days to heading and leaf rust severity but the lowest grain yield, aboveground biomass, and harvest index. At Adet, Cluster III emerged as the most prevalent, encompassing 29 genotypes, or 35.8% of the total, and Cluster IV with ten genotypes (12.3%). Clusters III and IV had the highest mean values for the grain-filling period,

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Table 11. Cluster membership profile of 81 barley genotypes based on 14 traits at Debark, Ethiopia

Cluster	Number of genotypes	Percentage (%)	Entries code
I	19	23.5	1, 5, 8, 12, 13, 14, 16, 17, 18, 24, 42, 44, 45, 51, 52, 63, 64, 69, 72
II	23	28.4	2, 3, 4, 10, 11, 19, 21, 22, 37, 38, 40, 47, 55, 56, 57, 58, 65, 71, 76, 78, 79, 80, 81
III	8	9.9	6, 7, 33, 34, 35, 49, 61, 66
IV	21	25.9	9, 15, 23, 27, 29, 32, 36, 41, 43, 46, 48, 50, 53, 54, 59, 60, 67, 68, 73, 74, 75
V	10	12.3	20, 25, 26, 28, 30, 31, 39, 62, 70, 77

Table 12. Means of the morphological traits in different clusters of barley genotypes at Adet and Debark, Ethiopia

Traits	Adet					Debark				
	I	II	III	IV	V	I	II	III	IV	V
Days to heading	71.1	80.6	61.9	61.7	64.8	78.5	80.3	74.8	72.8	73.2
Days to maturity	105.7	104.1	101.1	102.1	97.8	127.9	126.1	125.9	124.8	124.2
Grain filling	34.7	23.5	39.2	40.4	33.0	49.4	45.8	51.1	52.0	51.0
Plant height	100.8	70.1	96.2	105.2	104.6	116.5	110.3	117.4	106.3	118.5
Culm length	93.6	64.4	89.2	96.2	97.6	108.9	102.8	108.5	99.3	111.0
Awn length	13.5	10.4	11.8	12.5	12.8	12.7	12.6	13.3	12.1	13.2
Spike length	7.2	5.7	7.0	9.0	6.9	7.2	7.3	8.5	7.0	6.8
No. seed/spike	42.6	16.9	45.7	24.3	44.9	53.8	50.1	31.5	54.4	55.0
1000-seed weight	27.7	17.5	27.8	43.7	23.8	41.2	35.0	57.0	38.6	36.9
Dry biomass	8.7	2.8	9.8	10.1	6.8	19.6	16.4	18.6	17.7	16.8
Grain yield	2048	440	3189	2940	1307	6303	4767	5513	6600	4675
Harvest index	23.8	14.5	32.2	29.0	17.5	32.2	29.0	29.7	37.5	28.2
Leaf rust severity	14.4	82.5	12.5	11.3	71.8	9.9	26.5	15.6	13.0	33.5
Net blotch severity	3.7	4.0	2.9	3.6	1.4	2.9	3.1	4.3	2.5	1.6

harvest index aboveground biomass, and grain yield. However, Cluster III had the highest mean values for the number of seeds per spike, and Cluster IV had the highest mean values for plant height and thousand-seed weight. On the other hand, Cluster V included 11 genotypes (13.6% of the total) and had the highest mean leaf rust severity but the lowest mean grain filling period, harvest index, aboveground biomass, and grain yield.

Conversely, at Debark, as illustrated in Table 11, Cluster I included 19 genotypes, constituting 23.5% of the total, and was characterized by late maturation and high aboveground biomass with moderately greater plant height, thousand seed weight, seed per spike, and moderately high-yield genotypes. In contrast, Cluster II at Debark was the most dominant, comprising 23 genotypes (28.4% of the total), with the highest mean value for days to heading, days to maturity, and leaf rust severity and the lowest mean values for the grain filling period, harvest index aboveground biomass, and grain yield. In Debark, Cluster III included eight plants, representing 9.9% of the total; these plants had the highest mean spike length, thousand-seed weight, and net blotch severity, with moderate aboveground biomass, harvest index, and grain yield. However, Cluster IV included 21 genotypes, representing 25.9% of the total experimental materials; these genotypes presented the highest mean values for the grain filling period, number of seeds per spike, harvest index, and grain yield. Finally, Cluster V included ten genotypes, representing 12.3% of the total genotypes; these genotypes had the highest mean values for plant height and several seeds per spike, and leaf rust severity had the lowest mean harvest index and grain yield.

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In this study, the distribution of barley genotypes across two locations and their grouping patterns indicates variations among the studied genotypes (Table 12). These variations could be instrumental in future barley breeding programs to develop high-yielding varieties of barley with desirable morphological traits or environmental conditions.

Intra and inter-cluster distances

Genetic distance is the measure of the genetic divergence between barley genotypes. It quantifies the genetic dissimilarity between groups of genotypes. It provides a numerical value indicating the genetic change or evolutionary divergence between populations or individuals. Eighty-one barley genotypes were assessed to investigate their genetic divergence at Adet and Debark in Table 13 and 14, respectively. Utilizing cluster analysis, identified five distinct clusters, unveiling substantial agro-morphological variations within these groups of genotypes. The inter-cluster distances surpassed the intra-cluster distances, signifying extensive genetic diversity among the evaluated genotypes.

The highest inter-cluster distance was exhibited by clusters II and IV (75.0) followed by clusters II and III (59.35) and clusters IV and V (59.2) at Adet. These clusters were genetically more divergent from each other than any other pairs of clusters. Notably, the maximum intra-cluster distance was recorded for cluster II (6.01), followed by cluster IV (4.18), Conversely, the minimum intra-cluster distance was observed in cluster I at Adet. The smallest inter-cluster distance was observed between Cluster I and III (10.07) at Adet. Enyew et al. (2019) supported this study, reporting a maximum inter-cluster distance of 59.51 between clusters III and VI and a minimum of 17.68 between clusters III and IV. This substantial genetic divergence among genotypes suggests significant potential for heterosis in crossbreeding due to extensive genetic variability.

The maximum intra-cluster distance was recorded for cluster III (4.68), followed by cluster V (4.18). Conversely, the minimum intra-cluster distance was observed in cluster II at Debark. At Debark, the highest inter-cluster distance was exhibited by clusters III and V (103.98) followed by clusters III and IV (82.14) and clusters I and III (71.84). These clusters were genetically more divergent from each other than any other pairs of clusters. The smallest inter-cluster distance was observed between Cluster I and IV (13.77) at Debark. The genotypes of these clusters were relatively close to each other compared to those grouped in other clusters. Generally, this divergence analysis showed the presence of high genetic divergence among the tested 81 barley genotypes. Genotypes within clusters demonstrating a high degree of divergence are considered promising for breeding purposes, as they are likely to yield more desirable materials, leading to significant genetic advancement. The genetic improvement through hybridization and selection depends upon the extent of genetic diversity between parents, as indicated in a study by Hailu et al. (2016b). The

Table 13. Average intra (diagonal and bold) and inter-cluster (off-diagonal) distances among five clusters at Adet, Ethiopia

	1	2	3	4	5
1	2.19				
2	45.02**	6.01			
3	10.07	59.35**	2.05		
4	37.44**	75.3**	39.51**	4.18	
5	25.45*	34.25**	22.5*	59.21**	3.99

Notes: x2 = 22.36 and 27.69 at 5% and 1% probability level respectively.

Table 14. Average intra (diagonal and bold) and inter-cluster (off-diagonal) distances among five clusters at Debark, Ethiopia

	1	2	3	4	5
1	2.90				
2	15.69	2.50			
3	71.84**	66.74**	4.63		
4	13.77	20.04	82.14**	2.70	
5	24.44**	23.19*	103.98**	21.24	4.18

Notes: x2 = 22.36 and 27.69 at 5% and 1% probability level respectively.

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highest inter-cluster distance was observed between clusters II and IV (145.03), followed by clusters II and V (93.53), indicating that genotypes within these clusters exhibited relatively higher diversity than others. In contrast, the minimum distance occurred between clusters I and V (17.26). According to Jalata et al. (2020), 28 barley genotypes were evaluated 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 clusters II and III.

The intercluster values indicating close relationships suggest that hybridization among genotypes within these clusters may not result in a sufficient level of segregation. It is widely acknowledged that the greater the distance between clusters is, the broader the genetic diversity among the genotypes. Consequently, highly divergent genotypes possess the potential to generate a broad spectrum of segregation in subsequent generations, facilitating further selection and improvement. Hybrids developed from selected genotypes within the compatibility limits of these clusters may produce transgressive segregation exhibiting a high magnitude of heterosis. This approach is crucial for obtaining superior breeding materials with enhanced traits in subsequent generations. However, the breeder must specify his or her objectives to use best the traits where the traits are divergent.

The dendrograms from squared distances visually illustrate the complex relationships among diverse genotypes, highlighting varying degrees of resemblance within different clusters (Figures 4 A and B). This approach provides a holistic view of the genetic connections among the studied genotypes. Closer proximity in the dendrogram signifies greater similarity, indicating that genotypes near each other share more characteristics than those farther apart. The resemblance coefficient, which represents the value at which branches merge, measures the similarity between two genotypes.

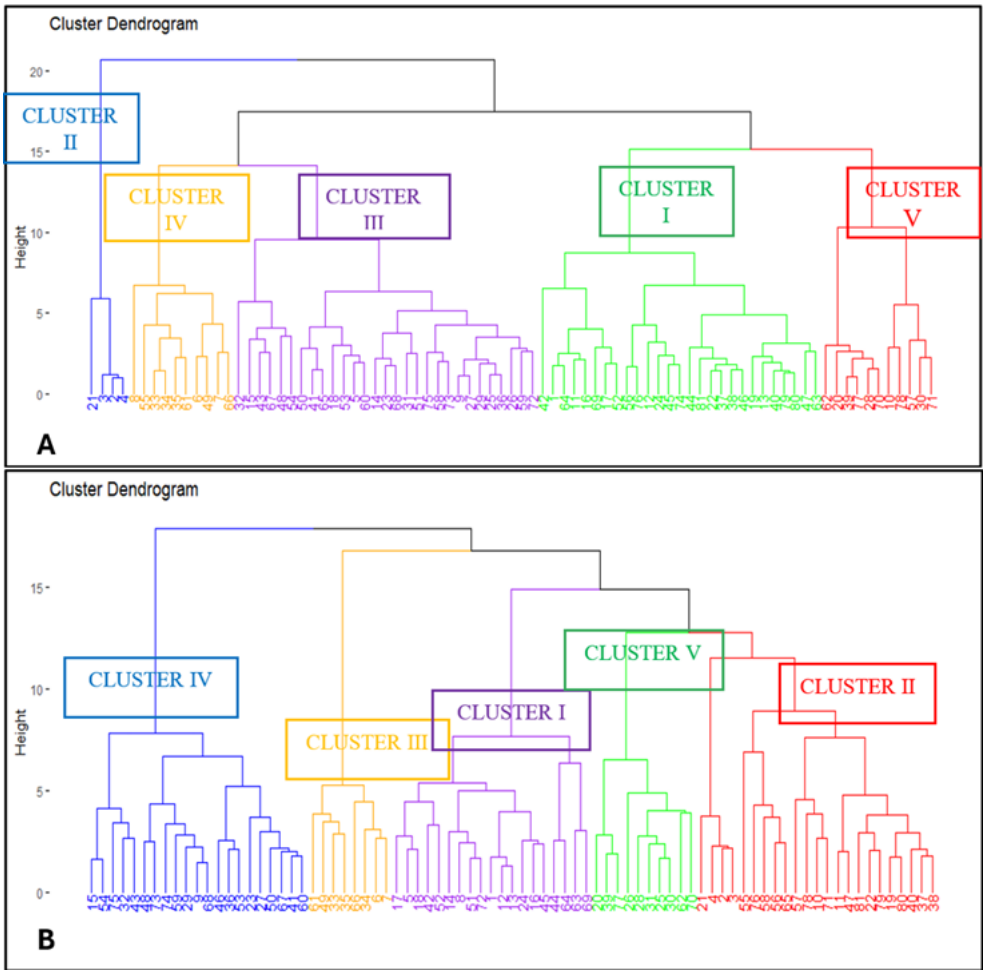

Figure 4. Dendrogram of 81 barley genotypes based on 14 agro-morphological traits at Adet (A) and Debark (B), Ethiopia

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Conclusion

This study provides a comprehensive assessment of genetic diversity and trait associations within barley genotypes cultivated at Adet and Debark, with objectives focused on exploring agronomic trait linkages, classifying genotypes based on genetic similarity and performance, identifying distinct genotype clusters, determining key traits driving genetic variability, and selecting promising barley genotypes. Analysis of variance for quantitative traits revealed significant genotype variations at both locations, indicating valuable genetic resources that could be harnessed in breeding superior cultivars. Principal component analysis at Adet showed the first four components explained 78.4% of genetic variation, with traits like grain filling period, plant height, aboveground biomass, and grain yield as primary contributors. In contrast, at Debark, five components accounted for 80.5% of the variation, emphasizing the roles of grain yield, grain filling period, harvest index, and thousand seed weight. Cluster analysis grouped genotypes into five clusters at each location, with notable inter-cluster distances indicating substantial genetic divergence. At Adet, clusters II and IV had the highest inter-cluster distance (75.0), suggesting strong genetic variation, whereas at Debark, the largest distance was between clusters III and V (103.98). Such distances indicate the potential for developing high-performing hybrids from highly divergent clusters. Genotypes 5, 9, 18, 32, 41, 50, 51, 53, 54, 60, 65, 68, and 75 exhibited promising yields, reaching 7291.6 kg.ha-1at Debark and 4631.85 kg.ha^{-1} at Adet. However, since it is a one-year result, it is necessary to repeat the field experiment for more years and over locations to make concrete conclusions and recommendations about the observed performance of genotypes. With further validation, these findings could be useful for barley breeding initiatives and have broader implications for agricultural productivity, economic stability, and food security.

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