Assessment of Genetic Divergence in Naked Barley (Hordeum vulgare L.) Genotypes Using Cluster and Principal Component Analysis

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Abstract

Understanding genetic diversity in crops is essential for developing effective breeding, conservation, and sustainable production strategies. This study evaluated 64 naked barley (*Hordeum vulgare*L.) genotypes at Bale Robe, southeastern Ethiopia, during the main cropping season using an alpha lattice design with two replications. Fifteen agronomic traits were recorded to determine the extent of genetic variation. Analysis of variance indicated significant differences among the genotypes for all the traits studied. Grain yield ranged from 2.915 to 6.035 t ha^{-1}, with accession 4138 achieving the highest yield and landrace 206306 the lowest. Principal component analysis identified four major components PC1 (38%), PC2 (19.8%), PC3 (13%), and PC4 (7.9%) with eigenvalues above one (5.7, 2.97, 1.96, 1.19), together explaining 78.8% of the total variation. Cluster analysis grouped the genotypes into four distinct clusters, with the greatest genetic divergence observed between clusters III and IV. The findings highlight the existence of substantial genetic diversity in naked barley, providing valuable opportunities for breeding programs aimed at enhancing yield potential and ensuring sustainable barley production in Ethiopia.

Introduction

Barley (*Hordeum vulgare* L.) is one of the world's oldest and most important cereal crops, cultivated across diverse agroecological regions for both food and feed purposes. It ranks fourth globally after maize, wheat, and rice in terms of production and area coverage. In Ethiopia, barley holds special cultural and economic significance, serving as a staple crop particularly in highland regions where other cereals perform poorly. The country is widely recognized as a primary center of origin and diversity for barley, possessing extensive morphological and genetic variability among its landraces (Lakew & Assefa, 2011)

The genus *Hordeum* consists of 45 taxa and 32 species, including diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploidy (2n = 6x = 42) cytotypes based on a basic chromosome number of seven (Bothmer & Jacobsen, 1985). Ethiopia, as a key diversity hotspot, harbors a wide array of barley landraces adapted to varied environments and traditional farming systems (Lakew et al. 1997). According to the Central Statistical Agency of Ethiopia (2022), the country is the leading barley producer in Africa, ranking fifth among cereals after maize, wheat, teff, and sorghum.

Barley's utilization in Ethiopia extends far beyond its agronomic importance. It plays a central role in traditional diets and cultural practices. The grain is processed into a range of food products such as *Injera*, *Dabbo* (bread), *Kitta/Torosho*, *Shorba* (soup), *Besso*, *Genfo*, *Chiko*, *Kinche*, and *Atmit/Muk* (Molla & Abebaw, 1998; Abebe et al, 2010). It is also an indispensable ingredient in local beverages such as *Tella* and *Areki*. Particularly, naked barley is preferred for the preparation of *Kolo*, a roasted grain snack consumed alone or mixed with roasted legumes (*Pisum sativum* L., *Cicer arietinum* L.) and oilseeds (*Guizotia abyssinica* L., *Carthamus tinctorius* L.) (Molla & Abebaw, 1998).

Despite its importance, barley production in Ethiopia faces multiple constraints, including poor adaptability to changing climatic conditions, low tolerance to abiotic stresses such as drought, lodging, and frost, and susceptibility to major diseases such as scald (*Rhynchosporium secalis* Oud.), net blotch

(*Helminthosporium teres* Sacc.), spot blotch (*Helminthosporium sativum* Pum.), and leaf rust (*Puccinia hordei* Otth.). Additionally, insect pests like the Russian wheat aphid (*Diuraphis noxia*) and shoot fly continue to limit productivity (Assefa, 2003; Tetyannikov & Bome, (2020)). These biotic and abiotic stresses collectively contribute to yield instability and threaten the conservation of valuable genetic resources (Bekele et al. 2005).

Although large numbers of barley accessions have been collected and conserved in Ethiopia, many remain inadequately characterized for key agronomic and morphological traits. Comprehensive evaluation of this genetic diversity is essential to identify promising genotypes for crop improvement and sustainable utilization (Abtew, 2020). Such characterization not only enhances breeding efficiency but also supports conservation strategies to safeguard native germplasm under the pressures of climate change and agricultural modernization.

Therefore, the present study was conducted to assess the extent of genetic diversity among naked barley genotypes using multivariate approaches such as cluster and principal component analyses. The findings are expected to provide valuable insights for breeders and conservationists in selecting parental lines for breeding programs and in developing strategies for the sustainable utilization of Ethiopian barley genetic resources.

Materials and Methods

Description of the Study Area

The experiment was conducted at the Madda Walabu University research station, located in the Bale Zone of the Oromia region, Ethiopia, during the main rainy season of 2023. The site lies at 7°7'N latitude and 40°0'E longitude (7.117°N, 40.000°E) at an elevation of 2,480 meters above sea level, receiving an average annual rainfall of 1,100 mm.

Experimental Material and Design

A total of sixty-four naked barley genotypes were evaluated, including 62 accessions obtained from the Ethiopian Biodiversity Institute (EBI) and two local genotypes sourced from farmers (Table 1). The experiment was arranged in a 4 × 16 alpha lattice design with two replications. Seeds were sown manually with an inter-row spacing of 20 cm on plots measuring 0.3 m² (1.5 m × 0.2 m). Plot, block, and replication separations were maintained at 0.2 m, 1 m, and 1 m, respectively. A seed rate of 120 kg ha^{-1} was used, and recommended fertilizer rates of 150 kg ha^{-1} urea and 100 kg ha^{-1} NPS were applied.

Source of Plant Material

The study evaluated 64 naked barley (*Hordeum vulgare* L.) genotypes, including 62 accessions from the Ethiopian Biodiversity Institute and two local genotypes (‘Negele’ and ‘EH-1493’) obtained from farmers

in the Ambo area of Oromia, Ethiopia. The accessions originated from several Ethiopian regions including Amhara, Oromia, SNNP, Benishangul-Gumuz, and Tigray. Detailed information on accession numbers and collection locations is provided in Table 1.

Table 1
List of Barley genotypes used as planting material that was collected from different part of Ethiopia

No	Accession	Region	Zone	Woreda	Latitude	Longitude
1	1617	Amara	S/Shewa	D/Berhan	10-47-00-N	37-07-00-E
2	1621	Amara	S/Shewa	D/Berhan	09-38-00-N	39-27-00-E
3	1696	Oromiya	Arssi	Tena	07-50-00-N	39-34-00-E
4	1855	Oromiya	M/Shewa	Waliso& Goro	08-45-00-N	37-50-00-E
5	3552	B/Gumuz	Metekel	Wenbera	11-15-00-N	35-55-00-E
6	3567	B/Gumuz	Metekel	Wenbera	10-30-00-N	35-50-00-E
7	3639	Amara	S/Gondar	Gondar	13-10-00-N	37-54-00-E
8	3701	Amara	S/Gondar	Debark	13-05-00-N	37-52-00-E
9	3724	Amara	S/Gondar	Debark	13-06-00-N	37-53-00-E
10	3737	Amara	S/Gondar	Wegera	12-50-00-N	37-43-00-E
11	3739	Amara	S/Gondar	Wegera	12-47-00-N	37-41-00-E
12	3746	Amara	S/Gondar	Gondar	12-47-00-N	37-41-00-E
13	4138	Amara	D/Gondar	Este	11-29-00-N	37-57-00-E
14	4164	Amara	D/Gondar	Este	11-37-00-N	38-04-00-E
15	4182	Amara	D/Gondar	Este	11-37-00-N	38-04-00-E
16	4212	Amara	S/Wello	Wadla	11-38-00-N	38-53-00-E
17	4395	Amara	M/Gojam	Sekela	10-59-00-N	37-10-00-E
18	4442	SNNP	S/Omo	Gofa	06-03-00-N	36-53-00-E
19	4476	Oromiya	M/Shewa	Cheliya	09-06-00-N	37-24-00-E
20	4490	Oromiya	M/Shewa	Cheliya	09-08-00-N	37-19-00-E
21	4500	Oromiya	M/Wellega	J/Horo	09-32-00-N	37-04-00-E
22	4533	Oromiya	M/Wellega	J/Horo	09-33-00-N	37-02-00-E
23	4605	Amara	M/Gojam	E/Enawga	10-45-00-N	38-04-00-E
24	4606	Amara	M/Gojam	E/Enawga	10-46-00-N	38-03-00-E
25	4658	Amara	M/Gojam	Guzamn	10-32-00-N	37-47-00-E
26	4752	Amara	S/Shewa	Mojana	09-57-00-N	39-51-00-E

No	Accession	Region	Zone	Woreda	Latitude	Longitude
27	4762	Amara	S/Shewa	D/Berhan	09-48-00-N	39-43-00-E
28	16922	Oromiya	Arssi	Limu&Bilbilo	07-25-07-N	39-13-41-E
29	16931	Oromiya	Arssi	Limu&Bilbilo	07-27-02-N	39-12-46-E
30	16946	Oromiya	Arssi	Limu&Bilbilo	06-34-06-N	39-16-54-E
31	17020	Oromiya	Arssi	Digeluna Tijo	07-41-38-N	39-15-59-E
32	17240	Amara	Agew Awi	Kosober	10-56-13-N	36-53-51-E
33	64080	Amara	S/Gondar	Debark	13-10-00-N	37-55-00-E
34	64164	SNNP	S/Omo	Chencha	06-19-00-N	37-28-00-E
35	64290	Oromiya	M/Wellega	Haru	09-36-00-N	37-50-00-E
36	64329	Oromiya	M/Shewa	Bako Tibe	09-04-00-N	36-36-00-E
37	64332	Oromiya	M/Shewa	Cheliya		
38	206306	Amara	D/Gondar	Kemekem		
39	206359	Amara	D/Gondar	Lay Gayint		
40	206366	Amara	D/Gondar	Lay Gayint		
41	206420	Amara	S/Gondar	Debark		
42	206423	Amara	S/Gondar	Debark		
43	206493	Amara	D/Gondar	Tach Gayint		
44	214219	Amara	S/Gondar	Debark		
45	214228	Amara	S/Gondar	Debark		
46	214229	Amara	S/Gondar	Debark		
47	214235	Amara	S/Gondar	Debark		
48	214797	Amara	D/Wello	Kalu		
49	215204	Oromiya	M/Shewa	Meta Robi	09-23-00-N	39-24-00-E
50	215223	Amara	S/Wello	Guba Lafto	11-48-00-N	39-26-00-E
51	215224	Amara	S/Wello	Guba Lafto	11-48-00-N	39-26-00-E
52	215580	Tigray	Debubawi	Ofla	12-13-00-N	38-43-00-E
53	219177	Oromiya	M/Harerge	Deder	09-15-00-N	45-20-00-E
54	219300	SNNP	Gedeo	Wenago		

No	Accession	Region	Zone	Woreda	Latitude	Longitude
55	219763	Amara	D/Gondar	Lay Gayint	11-44-00-N	38-20-00-E
56	228224	Amara	S/Wello	D/ Delant		
57	228228	Oromiya	M/Shewa	Wonchi		
58	243235	Oromiya	S/Shewa	Kembibit	09-36-19-N	39-29-50-E
59	244772	SNNP	Kembata	Angacha		
60	244904	Oromiya	M/Wellega	G/Horo		
61	244912	Oromiya	M/Wellega	G/Horo		
62	206417	Uknown	Uknown	Uknown		
63	Negele	Oromia	M/Shewa	Ambo		
64	EH-1493	Oromia	M/hewa	Ambo		

Data Collection

Field and laboratory data were collected in accordance with the International Barley Descriptor List (IPGRI, 1994), encompassing both plant-based and plot-based traits. Data were recorded from representative samples within each experimental plot to ensure reliability and accuracy. For quantitative traits such as grains per spike, grain weight per spike, spikelets per spike, peduncle length, plant height, productive tillers per plant, spike length, and total tillers per plant, observations were taken from ten randomly selected and individually tagged plants per plot. Each measurement was averaged to obtain a representative value for that plot. Grain yield per plot was determined by harvesting and threshing all plants within the net plot area. The total grain weight was recorded after proper cleaning and drying to approximately 12% moisture content. The plot yield was then converted to grain yield per hectare (kg ha^{-1}) using the formula:

$$GrainYield(kg/ha) = \frac{TotalGrainWeight\,(kg)}{PlotArea\,(m2)} x10{,}000$$

Other traits, including biomass yield, days to heading, days to maturity, grain filling period, grain yield, harvest index, and thousand-grain weight, were recorded from the entire plot for each genotype. The harvest index (HI) was calculated as:

$$HarvestIndex = \frac{GrainYield}{TotalBiomassYield}$$

,

Thousand-grain weight (TGW) was measured as the weight of 1,000 grains in grams. In addition to the field data, laboratory analyses were performed for selected quality parameters, following standard procedures outlined in the barley descriptor manual. Environmental conditions such as rainfall, temperature, and soil characteristics of the experimental site were also recorded to support interpretation of genotype performance. All data were collected from three replications to minimize experimental error and to allow for robust statistical analysis in subsequent multivariate evaluations.

Data Analysis

Data analysis was carried out to evaluate the extent and pattern of genetic diversity among the naked barley genotypes based on the measured quantitative traits. Prior to multivariate analyses, all trait data were standardized to a mean of zero and variance of one to eliminate the effects of scale differences among variables.

Results and Discussion

Analysis of Variance

Analysis of variance revealed highly significant differences ($p < 0.05$) among the 64 naked barley genotypes for all 15 agronomic traits, indicating the presence of substantial genetic variability within the evaluated population. Grain yield ranged from 2.915 to 6.035 t ha^{-1}, with accession 4138 recording the highest yield and landrace 206306 the lowest. Similarly, wide variation was observed for other quantitative traits such as plant height, spike length, productive tillers per plant, and grain weight per spike, reflecting the diverse genetic backgrounds of the genotypes. This phenotypic variability suggests a broad genetic base, offering valuable opportunities for selection and improvement.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was conducted to identify the major components explaining the overall phenotypic variation and to determine the relative contribution of each trait to total diversity among the naked barley genotypes. Components with eigenvalues greater than one ($\lambda > 1$) were considered significant according to the Kaiser criterion, and their corresponding loadings were used to determine the traits contributing most to genotype differentiation.

PCA grouped the evaluated traits into four principal components with eigenvalues greater than one ($\lambda > 1$). The first four componentsPC1 (38.0%), PC2 (19.8%), PC3 (13.0%), and PC4 (7.9%) together accounted for 78.8% of the total phenotypic variation among the genotypes (Table 2). The corresponding eigenvalues were 5.70, 2.97, 1.96, and 1.19, respectively. The first two principal components contributed most to the overall diversity, highlighting the key traits responsible for genotype differentiation.

PC1 (38.0%) exhibited strong positive loadings for spike length, spikelets per spike, grain weight per spike, thousand-grain weight, biomass yield, and grain yield, indicating that these traits were the major contributors to total variation and are essential determinants of yield potential. PC2 (19.8%) captured variation in phenological traits, particularly days to heading and days to maturity, while PC3 (13.0%) was influenced mainly by tiller number. PC4 (7.9%) reflected variation in grains per spike, plant height, harvest index, and peduncle length. Traits with higher absolute loadings on PC1 exerted a stronger influence on clustering, suggesting that genotype differentiation was driven by multiple traits rather than isolated characteristics (Chahal and Gosal, 2002).

These findings are consistent with previous reports (Enyew et al. 2020; Shtaya and Abdallah, 2019; Angassa and Mohammed, 2021), which indicated that the first four principal components explained 75–81% of the total variation in barley genotypes and were largely influenced by similar yield-related and morphological traits.

Table 2
Vector loadings and percentage of explained variation by the first four Principal Components

Variables	PC1	PC2	PC3	PC4
DH	0.07	0.49	0.18	0.18
GFP	-0.09	0.47	-0.04	-0.06
SL	0.30	0.11	0.10	0.00
NSPS	0.35	0.00	0.11	0.14
ProTil	0.09	0.17	-0.64	-0.08
ToTil	0.09	0.18	-0.64	-0.09
PH	0.24	0.22	0.18	-0.34
DM	0.00	0.53	0.13	0.14
PCL	0.13	-0.16	-0.03	-0.67
NGPS	-0.25	0.29	0.04	-0.30
GWPS	0.37	-0.03	0.02	0.16
TGW	0.39	-0.09	-0.07	0.14
BY	0.39	0.02	0.03	-0.06
GY	0.35	0.01	-0.11	0.14
HI	-0.23	-0.08	-0.23	0.44
Eigenvalue	5.70	2.97	1.96	1.19
Variability (%)	37.97	19.81	13.05	7.94
Cumulative (%)	37.97	57.78	70.83	78.77

Foot Note

BY= Biomass yield, DHA=Days to heading, DM=Days to maturity, GFP= Grain filling period, GWPS= Grain weight spike^{-1}, HI= Harvest index, NGPS=Number of grain spike^{-1}, NSPS=Number of spikelet spike^{-1}, PCL= Peduncle length, PH=Plant height, ProTil= Productive tiller plant^{-1}, SL =Spike length, TGW = Thousand grain weight, ToTil= Total tiller plant.

A PCA biplot (Fig. 1) was generated to visualize correlations between genotypes and quantitative traits. Genotypes are represented as blue points, while red arrows denote traits. Longer arrows indicate stronger contributions to the principal components. Traits such as peduncle length, biomass yield, spikelets per spike, and grain weight per spike aligned closely with Dim1, indicating their primary

influence on horizontal variability. Conversely, days to maturity, grain-filling period, and grains per spike were more associated with Dim2, affecting vertical variability. Trait correlations are further reflected by the angles between arrows: acute angles (e.g., biomass yield vs. grain weight per spike) indicate strong positive correlations, whereas obtuse angles (e.g., grains per spike vs. biomass yield) suggest negative correlations. Genotypes located near the origin represent average trait performance, whereas those at extremes are outliers with distinct profiles. These findings confirm the relevance of key agronomic and phenological traits in distinguishing naked barley genotypes, providing critical insights for parental selection and breeding programs, including under stress conditions (Yadav et al.; Pour-Aboughadareh).

Cluster Analysis

Cluster Analysis (CA) was performed using the agglomerative hierarchical method based on Euclidean distance as a measure of dissimilarity among genotypes. The average linkage method (UPGMA) was employed to construct the dendrogram, grouping genotypes with similar trait performance. The number of clusters was determined by examining the fusion levels and corroborating the grouping pattern with PCA-based scatter plots to ensure consistency. All statistical and graphical analyses were conducted in **R** statistical software using the packages *cluster*, *factoextra*, *prcomp*, and *ggplot2* (R Core Team, 2023).

Cluster analysis grouped the 64 naked barley genotypes (62 accessions and 2 local checks) into four distinct clusters. Cluster I comprised the largest group, containing 50 genotypes (78.13%), followed by Cluster II with 9 genotypes (14.06%), Cluster III with 4 genotypes (6.25%), and Cluster IV represented solely by the local check genotype ‘Negele’ (1.56%). The highest inter-cluster distance was observed between Clusters III and IV, followed by I and IV, and II and IV, indicating considerable genetic divergence among these groups (Table 3). The clustering pattern highlights substantial genetic variability among the evaluated genotypes, providing a valuable basis for selecting genetically diverse parents for future hybridization and breeding programs.

Table 3
Distribution of 64 naked barley genotypes into their respective cluster

Cluster	No. of genotypes	Proportion %	Genotypes				
			4752	**3639**	**64290**	**4500**	**64080**
			1617	214229	206306	3552	1621
			206359	206420	219177	215224	215580
I	50	78.13%	64164	64329	4605	215223	244904
			64332	16946	214228	206366	4490
			206417	4762	4533	219763	4164
			244772	206493	215204	4476	214797
			17240	3746	3701	3724	214219
			4658	214235	16922	244912	4395
			243235	228228	244772	16931	1855
II	9	14.06%	219300	4138	17020	4212	4182
			4442	1696	206423	4606	
III	4	6.25%	3737	3739	3567	EH-1493	
IV	1	1.56%	Negele				

Cluster Visualization Based on Principal Components

The cluster visualization derived from principal component analysis (PCA) revealed four distinct groups of naked barley genotypes, corresponding well with the hierarchical clustering results (Fig. 2). The first two principal components-Dim1 and Dim2-explained 38.0% and 19.8% of the total variation, respectively, together accounting for 57.8% of the overall genetic variability among the genotypes.

Cluster 1 (red) exhibited the widest genetic dispersion, indicating high intra-cluster diversity and representing genotypes with broad genetic backgrounds and multiple favorable traits. In contrast, Clusters 2 (green) and 3 (blue) were relatively compact and well-separated, reflecting distinct and stable trait combinations-potentially corresponding to specific adaptive or morphological characteristics. Cluster 4 (purple) was represented by a small group of rare or unique genotypes, suggesting the presence of specialized or regionally adapted accessions that may harbor valuable alleles for stress tolerance or specific yield attributes.

A moderate overlap between Clusters 1 and 2 suggests partial genetic similarity or shared phenotypic traits among genotypes in these groups, which may be due to common ancestry or adaptive convergence. These clustering trends are consistent with the findings of Abebe et al. (2024), who

reported similar groupings among Ethiopian barley genotypes associated with yield and drought-resistance traits. Likewise, Zhang et al. (2024). observed comparable clustering patterns in global barley germplasm, linking genetic diversity to environmental adaptation and stress tolerance. Collectively, these results reaffirm the utility of PCA-based clustering for visualizing genetic relationships and for identifying diverse parental lines for targeted breeding programs.

3.5. Genetic Divergence Analysis

Genetic divergence among the 64 naked barley genotypes was assessed based on Euclidean distance matrices computed in R using the *cluster* package (Table 2). The analysis revealed varying degrees of genetic dissimilarity among the four clusters. The smallest genetic distance was recorded between Clusters I and III (11.42), followed by Clusters I and II (18.19), indicating relatively close genetic relationships among these groups. Moderate divergence was observed between Cluster I and Cluster IV (33.07). The highest inter-cluster distance was observed between Clusters III and IV (38.54), suggesting that Cluster IV is genetically distinct and contains genotypes with considerable variability relative to the others. It is well established that greater inter-cluster distances correspond to higher genetic divergence, which can be exploited in breeding to produce transgressive segregants and enhance heterosis (Kumar et al. 2020) In this study, the pronounced divergence of Cluster IV from Clusters I, II, and III suggests that crosses involving genotypes from Cluster IV are likely to generate substantial genetic recombination, resulting in enhanced heterotic expression in F_1 hybrids and broader variability in segregating F_2 populations. Conversely, crosses between genetically closer clusters, such as I and III or I and II, may yield more uniform progeny with narrower genetic variability (Fantahun et al. 2023)

Overall, the genetic distance analysis highlights substantial genetic variability among the naked barley genotypes studied, providing a valuable foundation for parental selection, hybridization strategies, and genetic improvement. These findings emphasize the importance of genetically distant parents particularly those from Cluster IV for developing superior and diverse barley cultivars suited to different agroecological conditions (Table 4).

Table 4
Inter-cluster distances between centroids for 64 naked barley genotypes

Cluster	I	II	III	IV
I	0			
II	18.19			
III	11.42	20.13		
IV	36.58	33.07	38.54	0

χ^2 = 23.68 and 29.14 at 5% and 1% probability level respectively

3.6. Cluster Mean Analysis

Analysis of mean trait values across the four clusters revealed significant genetic variation among the evaluated naked barley genotypes, consistent with prior studies. Cluster III exhibited the highest performance for grain yield (5.38 t ha^{-1}), biomass yield (17.48 t ha^{-1}), and number of grains per spike (54.45), indicating its superior productivity and potential for direct use in high-yielding cultivar development. Similar patterns were reported by Belay et al. (2024) who identified clusters with enhanced yield and biomass traits in Ethiopian barley genotypes. Cluster II showed the best performance for spike length (8.93 cm), thousand-grain weight (46.97 g), and productive tillers per plant (7.06), reflecting favorable grain development and vegetative growth. These findings are in agreement with Güngör and Türkoğlu (2024), who observed similar trait patterns among barley clusters. Cluster IV, while exhibiting the highest harvest index (36.66%), had lower overall grain yield, highlighting a trade-off between resource allocation and productivity, also noted by Güngör and Türkoğlu (2024). Cluster I showed relatively lower grain yield (4.23 t ha^{-1}) but a longer grain-filling period (38.05 days), suggesting potential for breeding programs targeting extended grain-filling duration or stress resilience.

These results underscore the utility of cluster mean analysis for identifying barley genotypes with desirable combinations of traits, including yield, biomass, and physiological efficiency. Such insights provide valuable guidance for parental selection in hybridization and for developing cultivars with improved productivity and adaptability (Table 5).

Table 5
Cluster mean of 15 quantitative traits for 64 Naked Barley genotypes

Variable	Cluster I	Cluster II	Cluster III	Cluster IV	Grand Mean
DH	69.12	66.83	77.75	59.00	68.18
GFP	38.05	32.67	38.13	34.00	35.71
SL	7.35	8.93	8.50	5.72	7.63
NSPS	19.30	25.89	22.80	14.70	20.67
ProTil	6.88	7.06	6.55	6.90	6.85
ToTil	7.40	7.57	7.08	7.50	7.38
PH	94.17	97.62	100.55	83.02	93.84
DM	123.59	117.17	134.50	104.50	119.94
PCL	6.58	8.16	6.03	9.52	7.57
NGPS	57.37	26.53	54.45	44.00	45.59
GWPS	1.19	1.83	1.90	1.43	1.59
TGW	34.97	46.97	45.78	44.00	42.93
BY	12.15	16.85	17.48	13.93	15.10
GY	4.23	5.36	5.38	4.88	4.96
HI	35.11	32.11	30.83	36.66	33.68

Foot Note

BY= Biomass yield, DHA=Days to heading, DM=Days to maturity, GFP= Grain filling period, GWPS= Grain weight spike^{-1}, HI= Harvest index, NGPS=Number of grain spike^{-1}, NSPS=Number of spikelet spike^{-1}, PCL= Peduncle length, PH=Plant height, ProTil= Productive tiller plant^{-1}, SL =Spike length, TGW = Thousand grain weight, ToTil= Total tiller plant^{-1}.

The dendrogram depicting the four clusters based on 15 agronomic variables using Euclidean distance is presented in Fig. 3. In the figure, genotypes within the same cluster are marked with similar colors, while different clusters are distinguished by distinct colors. Closely related genotypes are connected within the same cluster, and genotype codes corresponding to accessions are indicated along the bottom axis.

Discussion

The considerable genotypic variation observed across all measured traits highlights the broad genetic base of Ethiopian naked barley accessions. This diversity represents an invaluable resource for global barley improvement efforts, particularly in breeding for resilience to biotic and abiotic stresses. The magnitude of variation recorded in grain yield and yield-related traits indicates that Ethiopian naked barley constitutes an untapped reservoir of adaptive alleles, consistent with its status as one of the world's oldest centers of barley domestication. Comparable trends in high intra-population variability have also been reported in Asian and European landrace collections (Horsley et al., 2022; Wang et al., 2023), emphasizing that landrace populations continue to play a crucial role in broadening the genetic base of cultivated barley.

Principal Component Analysis (PCA) revealed that a limited number of principal components explained most of the total phenotypic variability, with yield- and tillering-related traits exerting the strongest influence on genetic divergence. This pattern agrees with findings from large-scale germplasm evaluations in China and the Himalayas (Zhang et al., 2023; Chodron et al., 2024), where similar traits were identified as key discriminators among genotypes. Importantly, our analysis provides new evidence that Ethiopian naked barley exhibits trait interrelationships like those observed in global barley panels, thereby supporting its potential inclusion in international pre-breeding programs. The strong positive association between grain yield, productive tillers, and biomass yield suggests that these parameters can serve as efficient selection indices for yield improvement under marginal environments.

Cluster analysis further confirmed the existence of pronounced genetic divergence, partitioning genotypes into four distinct groups with substantial inter-cluster distances. The wide separation between clusters III and IV suggests the potential for exploiting heterosis through strategic hybridization between genetically distant genotypes. Similar diversity patterns have been documented among Tibetan and Central Asian naked barley accessions (Zeng et al., 2023; Chodron et al., 2024), underscoring the parallel adaptive diversification of barley under contrasting highland environments. The present study therefore contributes novel comparative insight by demonstrating that Ethiopian naked barley, though geographically isolated, exhibits genetic structures and trait associations comparable to other ancient barley centers of diversity.

Overall, these findings highlight the international relevance of Ethiopian naked barley as a unique genetic resource for global barley improvement. Despite being based on a single growing season, the study provides a valuable baseline for future multi-environment and genomic validation studies. The identification of distinct genetic clusters and trait-based principal components offers a clear framework for selecting parental lines that can contribute to the development of high-yielding, stress resilient barley cultivars suited to diverse agroecological conditions worldwide.

Conclusion

The present study revealed considerable genetic variability among 64 naked barley (*Hordeum vulgare* L.) genotypes for all evaluated agronomic traits. The significant differences observed across traits such as

grain yield, productive tillers, and spike morphology confirm the existence of a broad genetic base within the Ethiopian naked barley germplasm. Principal component analysis identified a small number of key components explaining the majority of total variation, with grain yield, biomass yield, and productive tillers emerging as major contributors. Cluster analysis further demonstrated clear genetic differentiation, grouping the genotypes into four distinct clusters with varying inter-cluster distances. These results collectively underscore the potential of Ethiopian naked barley accessions as valuable genetic resources for breeding and genetic improvement. Genotypes from highly divergent clusters particularly those showing superior performance for yield and yield-related traits should be prioritized as parents in hybridization programs to maximize heterosis and broaden the genetic base of future breeding populations. Moreover, the identification of diverse and high-performing genotypes supports targeted selection for specific environments, aiding the development of cultivars better adapted to the country's varied agroecological zones. Continued characterization of these landraces using molecular markers and multi-environment field trials will further enhance their utilization in sustainable barley improvement and conservation strategies.

Declarations

Author Contributions

Koricho Lemma designed and conducted the study, collected and analyzed the data, and drafted the manuscript. Chandra Sekhar Singh, Kumud Kant Awasthi, & Garima Awasthi critically reviewed the manuscript, provided suggestions, and assisted with data analysis. All authors read and approved the final version of the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Permissions to Collect Plant Material

The barley germplasm used in this study included 62 accessions obtained from the Ethiopian Biodiversity Institute (EBI), Ethiopia, and two local genotypes collected from farmers in the Ambo area of the Oromia region. The plant materials were provided for research purposes and their use complied with relevant national regulations and institutional guidelines of Ethiopia. No endangered or protected species were involved.

Plant Ethics / Collection Guidelines (Ethics and Consent to Participate)

All plant materials used in this study were cultivated barley genotypes obtained from recognized germplasm collections and local farmers. Their use complied with national and institutional guidelines for plant genetic resource research in Ethiopia. No protected or endangered species were used.

Competing Interests

The authors declare no competing interests.

References

1. Abebe K. Cluster and principal component analysis for yield and yield-related traits of food barley (*Hordeum vulgare* L.) genotypes at Woreilu district, South Wollo, Ethiopia. Int J Crop Sci. 2024;59(4):202–15.
2. Abebe TD, Bauer AM, Léon J. Morphological diversity of Ethiopian barleys (*Hordeum vulgare* L.) in relation to geographic regions and altitudes. Hereditas. 2010;147(4):154–64. ttps://doi.org/10.1111/j.1601-5223.2010.02173.x.
3. Abtew WG. 2020. Characterization of genetic variation among Ethiopian barley *(Hordeum vulgare L.) genotypes* [Master's thesis, Jimma University].
4. Angassa D, Mohammed J. Agro-morphological variability study of Ethiopian barley (*Hordeum vulgare* L.) accessions for their important agronomical traits at Hadiya Zone, Southern Ethiopia. J Plant Sci. 2022;10(1):19–26. ttps://doi.org/10.11648/j.jps.20221001.13.
5. Assefa A. 2003. Genetic variability and breeding potential of barley *(Hordeum vulgare L.)* landraces from North Shewa in Ethiopia [Unpublished master's thesis]. Addis Ababa University.
6. Bekele B, Alemayehu F, Lakew B. 2005. Food barley in Ethiopia. In S. Grando & H. Gomez Macpherson, editors, Food barley: Importance, uses and local knowledge,53–82. CABI Publishing. https://cabidigitallibrary.org.
7. Belay F, Alemu A, Abate Z. Cluster and principal component analysis for yield and yield-related traits of barley genotypes. J Agricultural Sci Technol. 2024;26(1):45–59.
8. Bothmer VR, Jacobsen N. 1985. Origin, taxonomy, and related species. In D. C. Rasmusson, editor, Barley (pp. 19–56). American Society of Agronomy. ttps://doi.org/10.2134/agronmonogr26.c2
9. Central Statistical Agency of Ethiopia. 2022. Production, area and yield by crops in Ethiopia. Public Knoema Data Hub. https://public.knoema.com/xnghehb/production-area-and-yield-by-crops-in-ethiopia
10. Chahal GS, Gosal SS. Principles and procedures of plant breeding: Biotechnology and conventional approaches. Alpha Science International; 2002.
11. Chodron N, Tashi T, Dorji T, Yangzom P, Wangmo K, Dorji S. 2024. Abundant genetic diversity harbored by traditional naked barley varieties on Tibetan Plateau: Implications in their effective conservation and utilization. *Biology*, 13 (12), 1018. https://www.mdpi.com/2079-7737/13/12/1018

12. Enyew M, Dejene T, Lakew B, Worede F. Clustering and principal component analysis of barley (*Hordeum vulgare* L.) landraces for major morphological traits from northwestern Ethiopia. Int J Agricultural Sci Food Technol. 2019;5(1):58–63. ttps://doi.org/10.17352/2455-815X.000043.
13. Fantahun B, Woldesemayate T, Fadda C, Gebrehawaryat Y, Pe E, Dell'Acqua M. Multivariate analysis in the dissection of phenotypic variation of Ethiopian cultivated barley (*Hordeum vulgare* ssp. vulgare L.) genotypes. Cogent Food Agric. 2023;9(1):2157104. ttps://doi.org/10.1080/23311932.2022.2157104.
14. Güngör NB, Türkoğlu N. GT biplot and cluster analysis of barley (*Hordeum vulgare* L.) genotypes based on agro-morphological traits. Agronomy. 2024;14(10):2188. ttps://doi.org/10.3390/agronomy14102188.
15. Horsley RD, Li C, Tinker NA. Genetic diversity and association mapping in global barley germplasm collections. Theor Appl Genet. 2022;135(2):455–69.
16. IPGRI. Descriptors for barley *(Hordeum vulgare L)*. International Plant Genetic Resources Institute; 1994.
17. Kumar Y, Niwas R, Nimbal S, Dalal MS. Hierarchical cluster analysis in barley genotypes to delineate genetic diversity. Electron J Plant Breed. 2020;11(3):742–8. ttps://doi.org/10.37992/2020.1103.122.
18. Lakew B, Assefa A. 2011. Advances and experiences in barley landrace improvement in Ethiopia. In Proceedings of the 2nd National Barley Research and Development Review Workshop (pp. 31–46). Holetta Agricultural Research Center.
19. Lakew B, Semeane Y, Alemayehu F, Gebre H, Grando S, van Leur JA, Ceccarelli S. Exploiting the diversity of barley landraces in Ethiopia. Genet Resour Crop Evol. 1997;44(2):109–16. ttps://doi.org/10.1023/A:1008609201169.
20. Melaku W, Tesfaye T, Regassa F. In: Brush SB, editor. Keeping diversity alive: An Ethiopian perspective. Lewis; 2000.
21. Molla A, Abebaw M. Barley utilization in Ankober Wereda, North Shewa. In: Yirga C, Alemayehu F, Sinebo W, editors. Barley-based farming systems in the highlands of Ethiopia. Ethiopian Agricultural Research Organization; 1998. pp. 85–94.
22. Tetyannikov NV, Bome NA. 2020. Sources of characters useful for breeding in hulless barley. Proceedings on Applied Botany, Genetics and Breeding, 3 (2), 49–55. ttps://doi.org/10.30901/2227-8834-2020-2-49-55
23. Pour-Aboughadareh A, Mohammadi R, Ahmadi J, Mehrabi AA, Etminan A, Sididi M. Biplot analysis of agro-morphological traits in barley (*Hordeum vulgare* L.) genotypes under drought stress conditions. Agron J. 2023;115(2):524–38. ttps://doi.org/10.1002/agj2.21594.
24. R Core Team. 2023. R: A language and environment for statistical computing (Version 4.4.2) [Computer software]. R Foundation for Statistical Computing. https://www.r-project.org
25. Shtaya MJY, Abdallah JM. Assessment of phenotypic diversity of barley genotypes through cluster and principal component analyses. J Anim Plant Sci. 2021;31(5):1345–51. ttps://doi.org/10.36899/JAPS.2021.5.0336.

26. Wang X, Liu H, Zhang J. Genome-wide association analysis of yield-related traits in a diverse barley panel. Plant Breeding. 2023;142(3):345–58.
27. Yadav R, Kumari P, Sharma S. Multivariate biplot analysis of exotic barley genotypes based on agro-morphological variables. Plant Archives. 2022;22(1):302–9.
28. Zewodu A, Mohammed W, Shiferaw E. Analysis of genetic diversity and population structure of some Ethiopian barley (*Hordeum vulgare* L.) accessions using SSR markers. PLoS ONE. 2024;19(6):e0305945. ttps://doi.org/10.1371/journal.pone.0305945.
29. Zhang L, He Y, Xu R. Multivariate analysis of agronomic and morphological traits in Chinese barley germplasm. Crop Sci. 2023;63(1):75–86.
30. Zhang Y. Genetic diversity and environmental adaptation in barley: A clustering approach for stress resistance and yield traits. Crop Sci Technol. 2024;62(1):98–112.

Figures

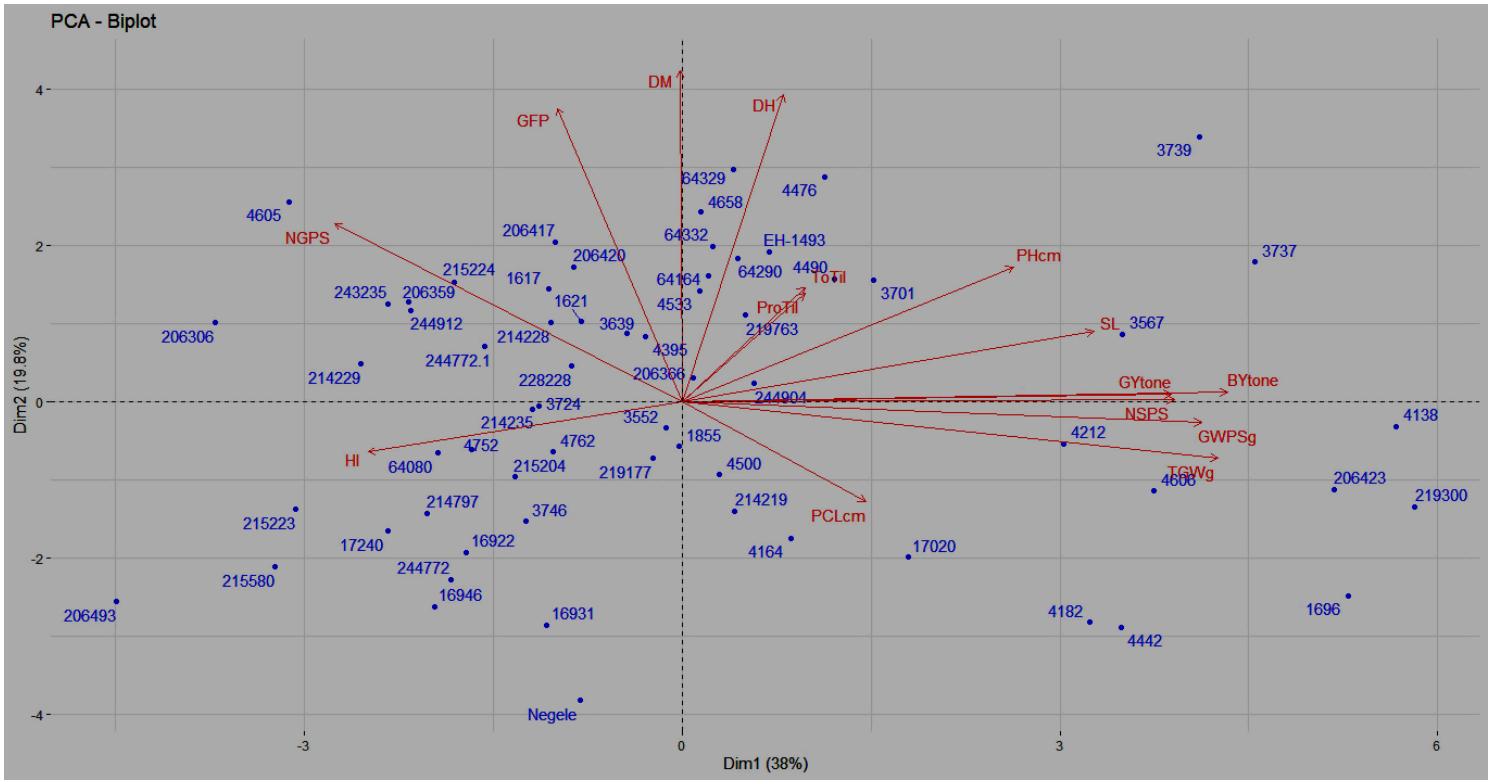

Figure 1

Bi plot of PC1 and PC2 showing the relationships of genotypes by traits. The blue color represents genotypes (n=64) and the red color represents the traits under study.

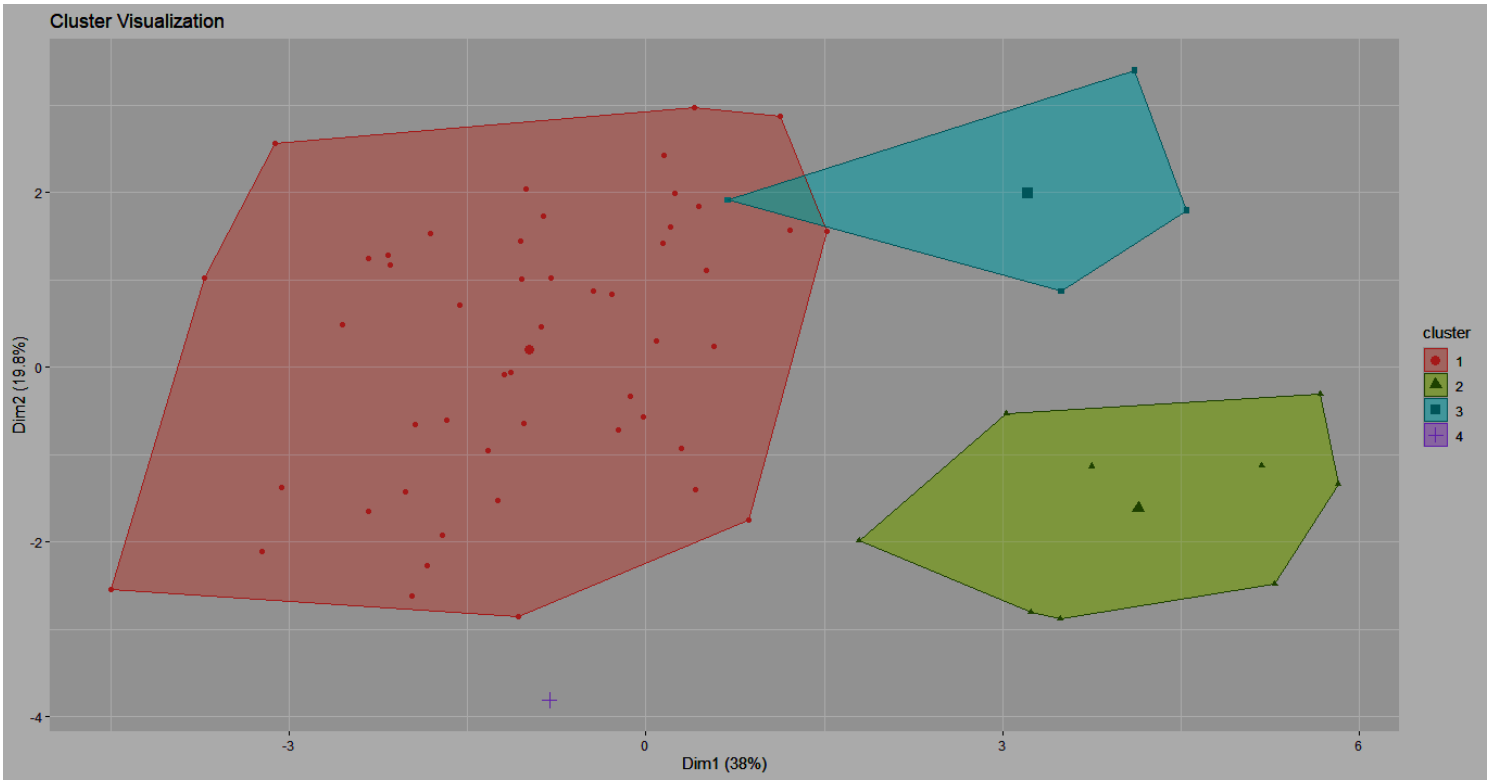

Figure 2

Cluster Visualization of 64 naked barley genotypes for 15 studied variables using Euclidean distance (average linkage method)

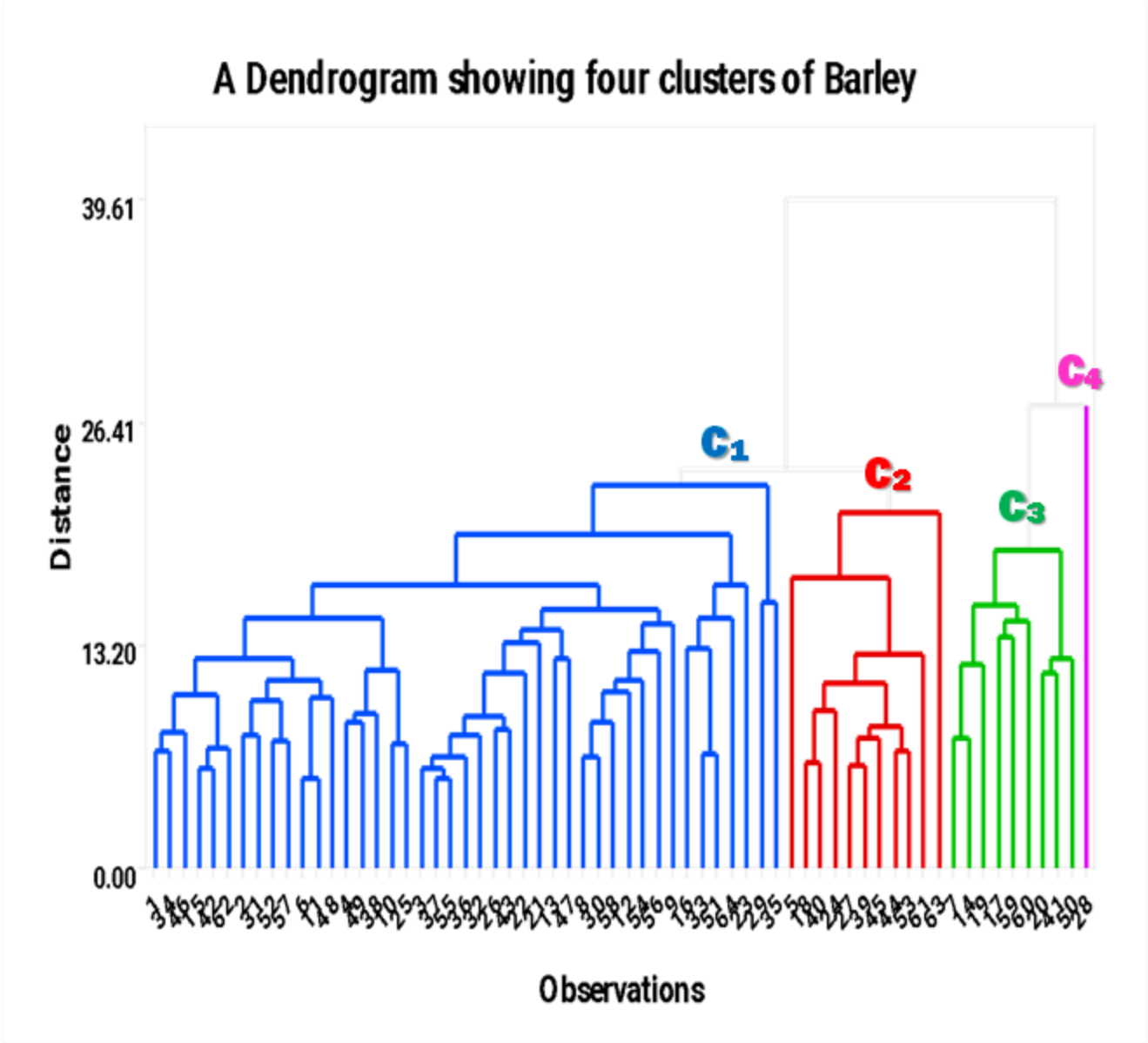

Figure 3

Dendrogram of 64 naked barley genotypes for 15 studied variables using Euclidean distance cluster analysis (average linkage method)

Supplementary Files

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- image1.png