

Dissecting the phenotypic diversity of Ethiopian barley (*Hordeum vulgare* L.) genotypes through variance components and multivariate analysis

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Abstract: Exploring the genetic diversity of barley germplasm conserved in the gene bank is critical for climate-resilient breeding. This study explored the phenotypic diversity and associations of 10 quantitative agro-morphological traits among 229 Ethiopian barley genotypes to identify desirable traits and promising accessions. The experiment was conducted at Holeta Agricultural Research Center in 2023 using an augmented design. The result of the analysis of variance revealed significant variation among the studied genotypes. A significant variability and wide range of mean performance were observed for the studied traits. Key traits like heading and maturity, grain filling period, 1000-kernel mass, kernel number per spike, and productive tillers were the most discriminating traits contributing to the highest variability (46.9 %). Cluster analysis identified three major trait-specific clusters. Grain yield exhibits a moderate correlation with plant height and kernel number per spike. High phenotypic and genotypic coefficients of variation were obtained for grain yield, productive tillers, kernel number per spike, and spikelet per spike. In addition, high heritability and genetic advance were observed for days to heading, productive tillers, spike length, kernel number and spikelet per spike, and grain yield suggesting selective breeding for these traits is likely to be effective for crop improvement programs.

Key words: barley, characterization, genetic variability, conservation, multivariate, augmented RCBD

Preučevanje fenotipske raznolikosti etiopskih genotipov ječmena (*Hordeum vulgare* L.) na osnovi variance in multivariatne analize

Izvleček: Preučevanje genetske raznolikosti ječmena shranjenega v genski banki je nujno potrebno za podnebno odporno žlahtnjenje. V raziskavi je bila preučevana fenotipska raznolikost in z njo povezane lastnosti desetih kvantitativnih agro-morfoloških lastnosti med 229 etiopskimi genotipi ječmena z namenom določiti željene lastnosti in obetavne akcesije. Izследki analize variance so pokazali značilne raznolikosti med preučevanimi genotipi. Med preučevanimi lastnostmi je bila ugotovljena značilna variabilnost z širokim razponom poprečij. Ključne lastnosti kot so čas do klasenja, zrelost, obdobje polnjenja zrnja, masa 1000-zrn, število zrn na klas, in število fertilnih poganjkov so se najbolj razlikovale in so največ prispevale (46.9 %). S klastersko analizo so bili prepoznani trije specifični grozdi, glede na glavne lastnosti. Pridelek zrnja je pokazal zmersno korelacijo z višino rastlin in številom zrn na klas. Doseženi so bili veliki fenotipični in genotipični koeficienti raznolikosti za pridelek zrnja, plodne poganjke, število zrn na klas in število klaskov na klas. Dodatno je bila opažena velika dednost in genetska prednost v znakih kot so število dni do klasenja, v številu plodnih poganjkov, v dolžini klasa, v številu zrn in klaskov na klas ter v pridelku zrnja, kar nakazuje, da bi bilo selektivno žlahtnjenje za te lastnosti verjetno učinkovito v programih izboljšanja te poljščine.

Ključne besede: ječmen, ovrednotenje, genetska spremenljivost, ohranjevanje, multivariatna analiza, izboljšani bločni poskus

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ABBREVIATIONS

CSA- Central Statistical Authority; DAP-Diammonium phosphate; EBI- Ethiopian Biodiversity Institute; GCV-Genotypic coefficient of variance; h^2 - Broad sense heritability; GAM- Genetic advance over mean; HARC- Holeta Agricultural Research Center; ICARDA-International Center for Agricultural Research for the Dry Areas; IPGRI-International Plant Genetic Resource Institute; m. a. s. l- meter above sea level; PCV- Phenotypic coefficient of variance; PCs- Principal components

1 INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the earliest domesticated crops that was originating from its wild progenitor *H. spontaneum* (K. Koch) Thell. in the Fertile Crescent ~10,000 years ago (Badr et al., 2000; Harlan and Zohary, 1966). It has been cultivated in a wide range of agroecologies from high altitudes to regions with low rainfall. In Ethiopia, barley was domesticated for more than 5000 years (Harlan, 1968) and is one of the fifth most important traditional food crops next to teff, wheat, maize, and sorghum in terms of area acreage and production (CSA, 2021). Barley cultivation covers more than one million hectares in Ethiopia and contributing to 8 % of the nation's total cereal production (Abtew, 2019; Kaso, 2015). Due to its short maturity duration relative to other crops, it is hugely consumed by small-scale farmers in the highlands of Ethiopia for poverty reduction and hunger alleviation during the lean period (Rashid et al., 2019). More than 80 % of barley grown by Ethiopian farmers is for food as compared to malt barley (Kaso, 2015). Besides, the straw is used for animal feed during the dry season and for thatching of roofs (Kaso, 2015). Cognizant of this, it is named as “*Gebis ye ehil nigus*” in highlanders of Ethiopia to mean “barley the king of all crops” (Mohammed et al., 2016).

Ethiopia is a secondary center of genetic diversity for its native two-rowed deficient and irregular barley types (Harlan, 1969; Vavilov, 1951). The Ethiopian barley landraces are diverse in morphology (two and six rows and irregular types) and color (black, white, and pink) (Asfaw, 1988) and also preferred by foreign breeders because of quality traits such as disease resistance, drought tolerance, high lysine, and protein content (Bjørnstad et al., 1997; Engels, 1991; Milner et al., 2019). It is also reported that the diverse in altitude, soil types, climates, topography, geographical isolation for long periods together with the farming system and wide socio-cultural diversity are the main driving forces for the diversity of barley in Ethiopia (Harlan, 1969; Tekle-

mariam et al., 2022). Like other cereals, barley production is heavily influenced by spatial and temporal variations in the environment (Dido et al., 2021) and thus exploring and harnessing the genetic diversity available in barley germplasm conserved in the gene bank is crucial for the improvement of the crop.

According to ICARDA (2025), around 400,000 barley accessions are conserved worldwide in gene banks, breeders, and research collections. In Ethiopia, barley has been conserved in-situ in community seed banks located in different agroecologies of the country and ex-situ in gene bank. For instance, Ethiopian Biodiversity Institute (EBI) has maintained the largest barley farmer varieties (> 17,000 (~4.25 %)) in its cold storage facilities as compared with other crops (<https://ebi.gov.et/biodiversity/conservation/genetic-material-holdings/>). In Ethiopia, farmer varieties or landraces constitute 90 % of the total land devoted to barley production (Hadado et al., 2009). In fact, landraces or farmer varieties exhibited both within and between variation as compared to the genetic uniformity of modern cultivars (Zhu et al., 2000). Thus, maintaining the genetic diversity in crops is required to sustain genetic improvement for polygenic traits, such as yield. Previous studies showed that landraces are the major constituent of useful genes for adaptation to biotic and abiotic stresses (Asfaw, 1988; Gagnaw & Hadado, 2014). Most specifically, recent studies by Megersa et al. (2015), Monteagudo et al. (2019), and Wosene et al. (2015) revealed that barley landraces have shown higher yield stability and comparable yield with improved varieties. As such, characterization, and evaluation of genetic variability between and within barley landraces is an essential component for sustainable conservation and improvement. Owing to these, numerous genetic diversity studies were carried out on Ethiopian barley in relation to altitude, eco-geographical distribution of isozyme and allozyme, and association of hordein and morpho-agronomic traits (Asfaw, 1988, 1989; Assefa et al., 2016; Bedasa et al., 2015; Engels, 1994; Fantahun et al., 2023b; Gadissa et al., 2021)

Multivariate analysis techniques such as principal component analysis (PCA), clustering, and correlation were commonly employed by researchers (Abebe et al., 2010; Angassa & Tesfaye, 2019; Derbew, 2020; Enyew et al., 2019; Fantahun et al., 2023a) to investigate the genetic relationship between genotypes and association of traits. Besides, genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV); heritability, and genetic advance were used by researchers (Addisu & Shumet, 2015; Kaur et al., 2022; Shiferaw et al., 2020; Shtaya et al., 2015) to explore genetic relationships between genotypes and

the association of traits. Despite the enormous efforts made by national and international programs to conserve barley diversity, there is, however, little information is yet available as compared with the existing huge genetic resources in Ethiopia which remained underutilization of germplasm accessions preserved in the gene bank. Therefore, a rigorous characterization and evaluation are utmost important to have sufficient characterized accessions to researchers and breeders. In this context, the present study was conducted to explore the genetic variability and association of yield and yield-related components among 229 barley genotypes and identify superior traits and accessions that can contribute to future barley grain yield improvement program and conservation.

2 MATERIAL AND METHODS

2.1 PLANT MATERIALS AND EXPERIMENTAL SITE

A total of 232 barley genotypes comprised of 229 accessions obtained from the Ethiopian Biodiversity Institute and three improved check cultivars containing six-rowed food barley (HB-1307 and HB-1966) and two-rowed malt barley (Suba) (Figure 1B; Additional File 1, Sheet 1) released by Holeta agricultural research center (HARC) were characterized. The accessions were requested from the gene bank owing to the major barley growing regions, altitudinal class (1940-3546 m. a. s. l) and previous characterization history in the gene bank. The study was conducted in 2022/23 main growing season at the research field of HARC located in the Oromia

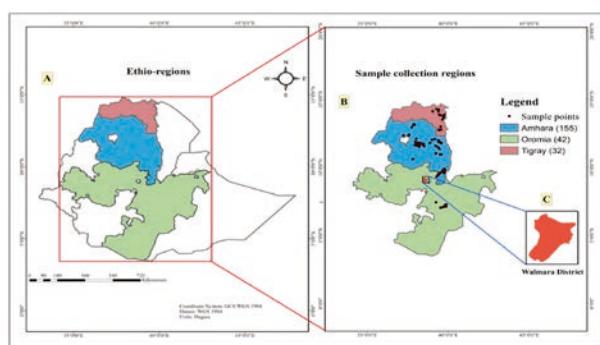


Figure 1: Map of Ethiopia showing the administrative regions (A), sample points where barley study materials were collected (B) and experimental site (C). Map was generated by ArcMap 10.2.2 (Esri, 2014). (All boundaries were prepared in accordance with Ethiopia Geoportal (Last updated on May 2020; <https://ethiopia.africageoportal.com/>)

regional state of West Shewa zone, Walmera woreda (Figure 1C) ($9^{\circ}00'00''$ N, $38^{\circ}30'00''$ E, elevation 2400 meter above sea level. The temperature varied from 6-22 °C with an average annual rainfall of 1144 mm and light soil (Nitrosols and Vertosols (<http://www.eiar.gov.et/holetta/>).

The experiment was laid out by an Augmented block design where the check cultivars were replicated and appeared once in each block but test treatments appeared once in the design. The design assumes checks as fixed effects whereas entries as random effects. The experimental area contained a total of seven blocks. Each block had a total of 36 entries comprised of 33 accessions and three checks. To scrutinize the bias among treatments, randomization were done by Agricolae package of R-software (Mendiburu, 2020). Each accession was drilled by hand in 4 rows of 2 m^2 having with 2.5 m row length with 0.2 m row spaced at 85 kg ha^{-1} seed rate (17 g plot $^{-1}$). The distance between each plot & block was 0.4 m & 1 m respectively. All the recommended agronomic practices were applied to each treatment equally before and after sowing according to the standard operational procedure of characterization (Ethiopian Biodiversity Institute, 2021). Artificial fertilizer was applied equally to each treatment before sowing at the rate of 36.4 g NPS and 11.2 g UREA split to 7.5 g (during sowing) and 3.7 g (during tillering after first weeding).

2.2 DATA COLLECTION

All data were electronically recorded through tablet using the FieldScorer Android App (<http://www.katmandoo.org/Help/Fieldscore4Android/index.html>) after developing a standardized comma delimited (csv) trial and trait files (Tamirat Bejiga and Amare Seyoum, 2018). The traits were prepared in accordance with the barley descriptor list developed by Bioversity International (IPGRI, 1994) with minor modifications. Since three accessions were missed due to heavy water stress, data were exclusively collected for 229 genotypes (226 accessions and three checks). Quantitative data were collected on a plant and plot basis after randomly selecting and tagging 20 representative plants from each plot and the average of these samples was used for the analysis. Three phenological and seven agro-morphological traits were recorded in this study. Days to heading (DTH) were counted as the number of days from sowing to 50 % of plants fully emerged (Z55 stage (Zadoks et al., 1974)); days to maturity (DTM) was counted as the number of days from sowing to 75 % of the plants physiologically matured); grain filling period (GFP = DTM - DTH) was measured as the number

of days from the time that half of the plants have started flowering to 75 % of the plants fully matured; plant height (PH, cm) was measured at full maturity from the ground level to the top of the spike excluding awns; productive tillers (PT) was counted on the number of effective tillers arising from the main plant; spike length (SL, cm) was taken from the neck to the base of the lemma awns of the uppermost grain; spikelet per spike (SPS) was counted as the number of spikelet raised from each node; kernel number per spike (KNS) was counted as the number of kernels obtained after threshing of spikes; thousand kernel mass (TKM, g) were measured after weighing and converting of 250 seeds and grain yield (GY, kg ha⁻¹) were obtained from the field plot (g m⁻²) after extrapolating the area of 2 m² to hectare basis. TKM and GY were determined after adjusting to 12.5 % seed moisture content.

2.3 STATISTICAL DATA ANALYSIS

2.3.1 Estimates of analysis of variance and genetic variability

The quantitative data obtained from augmented design were subjected to analysis of variance (ANOVA), and genetic variability analysis using augmented RCB bulk function of the augmented RCBD package (Aravind et al., 2021) in R software (Team, 2024). The phenotypic (), genotypic (and environmental variance (were estimated from the ANOVA tables according to the expected value of the mean square described by Federer and Searle (1976) as follows:

$$\text{Genotypic variance } (\sigma^2 g) = \frac{MSg - MSE}{r} \quad (1)$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \sigma^2 e \quad (2)$$

Where MSg-mean square due to the genotype, MSE-error mean square, r-number of replications for the check (control) genotypes, -Phenotypic variance, $\sigma^2 g$ -Genotypic variance, $\sigma^2 e$ - Environmental variance = error mean square (MSE). The phenotypic and genotypic coefficients of variation (PCV & GCV) were estimated according to (Burton, 1951) and categorized as low (< 10), medium (10-20) and high (≥ 20) according to (Sivasubramaniam S, 1973).

$$PCV = \frac{\sigma^2 p}{\sqrt{\bar{x}}} \times 100 \quad (3)$$

$$GCV = \frac{\sigma^2 g}{\sqrt{\bar{x}}} \times 100 \quad (4)$$

Where $\sigma^2 p$ = Phenotypic variance, $\sigma^2 g$ = Genotypic variance and \bar{x} = the grand mean of the trait under consideration.

Heritability (h^2) in the broad sense was estimated according to the method of (Lush, 1940) and categorized according to (Robinson, 1966) suggestion as low (< 30), medium (30-60) and high (≥ 60).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100 \quad (5)$$

Genetic advance (GA) and genetic advance as a percent of the mean (GAM) were calculated by assuming the selection of more than 5 % of the genotypes estimated per the methods illustrated by (Johnson et al., 1955).

where GA - expected genetic advance, σ_p - phenotypic standard deviation on the mean basis, h^2 - Heritability in a broad sense, K = selection differential (where k = 2.06 at 5 % selection intensity).

$$GA = k \times \sigma_p \times \frac{h^2}{100} \quad (6)$$

The genetic advance (as a percent of the mean) (GAM) was computed to compare the extent of the predicted genetic advance of different traits under selection and categorized as low (< 10 %), moderate (10 %–20 %), or high (> 20%) according to (Johnson et al., 1955)

$$GAM = \frac{GA}{\bar{x}} \times 100 \quad (7)$$

Where GAM - genetic advance as a percentage of the mean, - Grand mean of the quantitative character

2.3.2 Multivariate analysis

The adjusted mean values generated from the ANOVA analysis were subsequently used for the computation of principal component analysis, pairwise correlations, and clustering analysis. Prior to analysis, the adjusted mean values were standardized to means of zero and variances of unity to avoid variations in the scales used during data collection (Manly, 1986; Sneath and Sokal, 1973) using the scales function in R software (Wickham & Seidel, 2020). The principal component and biplot analysis was computed using FactoMineR (Lê et al., 2008), and ggbiplot (Vu, 2011). A Spearman rank correlation was generated by GGally (Schloerke et al., 2024) and ggplot2 (Wickham et al., 2016) packages. A phylogenetic tree-based cluster analysis was performed after determining the cut-off point using the NbClust package (Charrad et al., 2014). The cluster analysis was hierarchically catego-

rized by the Wards linkage clustering method. Besides, the genetic distance among genotypes and divergence among clusters was performed by using NbClust package (Charrad et al., 2014) and clv package (Nieweglowski & Nieweglowski, 2015) respectively.

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE (ANOVA)

The results of ANOVA presented in Table 1 showed a significant difference ($p \leq 0.05$) to highly significant ($p \leq 0.01$) difference among tests (accessions) and genotypes (entries) for PT, DTH, DTM, PH, SL, SPS, KNS, TGW, and GY. In contrast, a non-significant difference was observed for GFP. These notable significant differences among accessions revealed that substantial genetic variability exists which

can be harnessed through selection. In agreement with our study, Zewodu et al. (2025) reported highly significant differences ($p \leq 0.01$) for DTH, DTM, PH, SL, SPS, KNS, TCM, and GY. Similarly, (Derbew, 2020; Dido et al., 2020) also reported a non-significant difference for GFP and a highly significant difference for other parameters. The two-food barley and one-malt barley improved varieties used for standard checks showed significant ($p \leq 0.05$) variation for GFP, PH, and PT to highly significant variation ($p \leq 0.01$) for the rest of the parameters except DTH which showed non-significant variation. A similar study on 102 Ethiopian food barley landraces and five checks (Bedasa et al., 2015) reported highly significant variation for PH, SL, KNS, and TCM. In addition, the mean square due to test (accessions) v/s checks were highly significant ($p \leq 0.01$) for DTM, DTH, GFP, PH, and KNS traits indicating the presence of significant differences between landraces and checks in these traits.

Table 1: The mean square of ANOVA for 10 morpho-agronomic traits of studied barley genotypes (n = 229) resulted from augmented design

Sources	Mean squares [†]									
	DTH	DTM	GFP	PH	PT	SL	SPS	KNS	TKM	GY
Blocks (6)	4.98 ^{ns}	14.43 ^{ns}	11.94 ^{ns}	140.52 ^{**}	0.64 [*]	1.02 [*]	3.25 [*]	5.62 ^{ns}	5.91 [*]	1304592.5 ^{**}
Among genotypes (228)	95.73 ^{**}	158.17 ^{**}	24.09 ^{ns}	108.73 ^{**}	0.49 [*]	1.35 ^{**}	36.77 ^{**}	108.9 ^{**}	26.6 ^{**}	690112.7 ^{**}
Among check (2)	11.48 ^{ns}	147.57 ^{**}	76.76 [*]	136.92 [*]	2.59 ^{**}	6.53 ^{**}	185 ^{**}	951.9 ^{**}	64.3 ^{**}	951074.6 ^{**}
Among accessions (225)	92.43 ^{**}	143.71 ^{**}	20.52 ^{ns}	102.22 ^{**}	0.47 [*]	1.31 ^{**}	35.6 ^{**}	100.3 ^{**}	26.4 ^{**}	688720.7 ^{**}
Accessions vs. Check (1)	1006.8 ^{**}	3432.7 ^{**}	721.4 ^{**}	1517.7 ^{**}	0.3 ^{ns}	0.54 ^{ns}	3.53 ^{ns}	369.3 ^{**}	4.77 ^{ns}	481399.7 ^{ns}
Residuals (12)	4.37	17.9	12.65	21.8	0.16	0.33	0.96	3.56	1.91	136411.1
CV (%)	2.85	3.69	8.58	5.5	22.4	7.02	4.75	5.52	2.81	17.6
Standard Errors										
A Test Treatment and a Control Treatment	2.58	5.22	4.39	5.76	0.49	0.71	1.21	2.33	1.7	455.92
Control Treatment Means	1.12	2.26	1.9	2.5	0.21	0.31	0.52	1.01	0.74	197.42
Two Test Treatments (Different Blocks)	3.41	6.91	5.81	7.62	0.65	0.94	1.6	3.08	2.25	603.13
Two Test Treatments (Same Block)	2.95	5.98	5.03	6.6	0.56	0.82	1.39	2.67	1.95	522.32

† DTH-Days to 50 % heading, DTM-days to 75 % maturity, GFP-grain filling period, PH-plant height (cm), PT-productive tiller, SL-spike length (cm), SPS-spikelet per spike, TCM-thousand kernel mass (g) and GY-grain yield (Kg ha⁻¹); genotypes-accession + checks. ns p > 0.05, * p ≤ 0.05, ** p ≤ 0.01; CV- coefficient of variation; Numbers in parenthesis represented degree of freedom.

3.2 AGRO-MORPHOLOGICAL TRAIT DIVERSITY AND MEAN PERFORMANCE OF GENOTYPES

The mean performance of the studied barley genotypes for 10 morpho-agronomic traits is presented in Additional File 1, Sheet 2. In general, barley genotypes exhibited a high level of variation in yield and related yield components (Figure 2; Additional File 1, Sheet 2). Meanwhile, the mean value of DTH was 72.7 days and varied from 54 days to 99 days. On the other hand, the number of days to attain 75 % physiological maturity (DTM) had a mean of 113.7 days with a range of 89 days to 146 days. In line with our work, a combined two and six-row barley study by (Gadissa & Gudeta, 2023) and (Angassa & Tesfaye, 2019) showed a comparable DTH (56-97 & 56-93 days) and DTM (99-138 & 89-140 days) respectively. In contrast, a wide range of DTH (91-116 days) and DTM (117-174 days) were also reported on Ethiopian six-row barley genotypes by Alemayehu & Parlevliet (1997) and Megersa et al. (2015). In the present study, two-rowed barley accession-243314 had taken earlier heading (53 days) and maturing time (89 days) as compared to six-rowed barley accession-243209 (98 days and 146 days respectively) which is consistent with (Fantahun et al., 2023a; Kandić et al., 2018; Setotaw et al., 2010;

Zewodu et al., 2025) studies. These results, however, disagree with Kaur et al. (2022) study who reported as two-rowed varieties took many days to mature as compared to six-rowed varieties. GFP on the other hand is found to be a crucial determinant of the genetic variation in rice (Yang et al., 2008) which ranged from 31 days to 59 days with a mean of 41 days and CV of 8.58. In a similar study, a wide range of GFP (23.88-34.22) with a mean of 30.11 was reported by (Megersa et al., 2015). In general, the difference in the number of days (DTH, DTM, and GFP) observed in our study is mainly attributed from the genetic background of genotypes (two-rowed, six-rowed, irregular, and hulless) and the mixture of materials used. This is from the fact that the barley genetic materials preserved in the gene bank are in the population form.

The regional diversity of agronomic traits (Table 2) also revealed that accessions collected from the Amhara region showed minimum values of DTH (54 days), DTM (89 days), and GFP (31 days) as compared to the maximum values of DTH (99 days) and DTM (146 days) observed for accessions collected from Oromia and local improved varieties. In fact, earliness is one of the key adaptive traits as demonstrated in wheat (Hyles et al., 2020; Mondal et al., 2013) that enables crops to escape terminal moisture stress and ensuring more reliable yields under unpredictable and re-

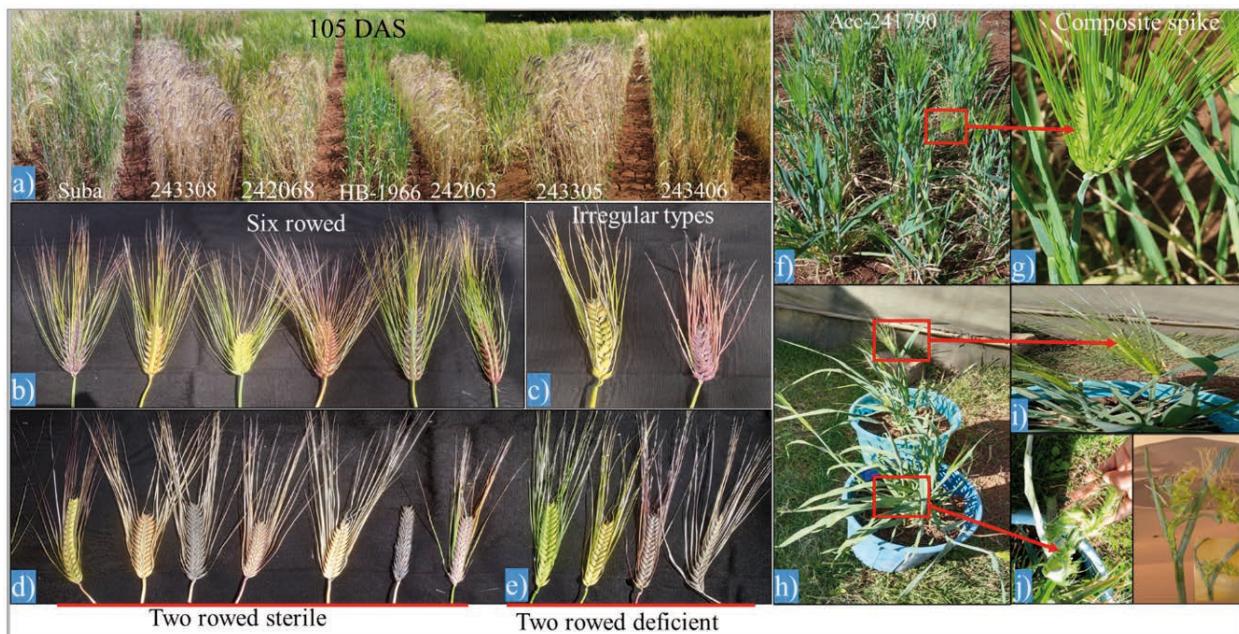


Figure 2: Diversity of some of barley genotypes based on maturity and grain filling period at 105 days after sowing (DAS) (a); row types i.e. six row (b) irregular types (c) two rowed sterile, long awn and awnless (d) two rowed deficient (e) hulless and composite barley (Acc-241790) (f&g) found in the field (top right corner) and a pot containing the same composite barley seeds planted in the Lathouse (h) and emerged two different spike forms i.e. a single spike (i) and triple-spikes (j)

source-limited conditions (Kandić et al., 2018; Megersa et al., 2015; Yadav et al., 2018) and hunger alleviation during the lean period (Amri et al., 2005; Rashid et al., 2019). Therefore, the wide range of variation present in the studied genotypes across regions for phenological traits allows breeders to improve grain yield for short and extended growing seasons accordingly.

Plant height (PH) is another important agronomic trait highly valued by farmers (for animal fodder, thatching roofs) and breeders (lodging tolerance) (Kaso, 2015; Kaur et al., 2022). In this study, the highest (108.4 cm) PH was found for accession 243296 and the lowest (57.8 cm) being for accession 241790 with a mean of 85.6 cm and CV of 5.5. Interestingly, accession 241790 (Figure 2f) is found to be six-rowed hulless and merely has composite spike forms with dwarf plant height (57.8 cm) and also a minimum grain filling period (32 days). To further elucidate the mode of inheritance, we planted the seed obtained from composite barley spike (Figure 2g) in two pots in Lathouse (Figure 2h) and it was found that > 99 % of the spikes that emerged were single spikes while only a single spike showed triple-spike form (Figure 2j). This stipulated that accession 241790 could have a potential poly-row-branch-spike (*prbs*) gene (Poursarebani et al., 2015, 2020; Terzi et al., 2017) which needs further investigation at the multi-location and genomic level.

Grain yield (GY) is the consequence of multiplicative interaction of intricate traits such as SL, SPS, KNS, PT, TKM, and rarely on PH (Dziurdziak et al., 2020; Kaur et al., 2022). In this study, a wide range of variation was observed for SL (5.2-11.5 cm), SPS (12.5-33.9), KNS (14.3-53.9), and TKM (31.9-62.4 g). Whereas, a narrow range of PT (0.23-3.7) with a mean value of 1.78 was observed for accession 243274 and 243307 respectively (Table 1). Previous studies (Angassa & Tesfaye, 2019; Dziurdziak et al., 2020, 2021) also reported a closer range and mean value of PH, SPS, KNS, SL and TKM. A comprehensive phenotypic characterization and genetic diversity analysis of 6,778 barley germplasm (Kaur et al., 2022) also revealed a wide variation in PH (45.96-171.32 cm), SL (3.44-13.73 cm), KNS (10.48-82.35) and TKM (12-68.6 g). Besides, the lowest GY (292.3 kg ha⁻¹) was recorded for two-rowed barley accession-243212 and the highest (3599.9 kg ha⁻¹) being for six-rowed accession-243313 with an average of 2118.3 kg ha⁻¹ (Additional File 1, Sheet 2). Derbew et al., (2013) reported a wide range of variation in GY (436-3752.5 kg ha⁻¹). It is evident from (Verma et al., 2021) study that two-rowed barley generally have a reduced number of grains per spike which is significantly affecting the final grain yield as compared to six-rowed ones. The regional diversity of morpho-agronomic parameters (Table 2) also indicated that accessions collected from Amhara have high SPS (50.5), TKM (62.9

g), and GY (3599.9 kg ha⁻¹) as compared to SPS (26.2), TKM (51.6) and GY (2225.8 kg ha⁻¹) of standard checks exploited in the study. In general, out of 229 barley genotypes, 119 (52 %) genotypes have given higher GY than the grand mean (2118.3 kg ha⁻¹) (Additional File 1, Sheet 2). Despite the poor attitude towards landraces on their low yield potential (Azeez et al., 2018), the average GY obtained in this study, however, is significantly higher than the improved varieties HB-1307 (2102.9 kg ha⁻¹), HB-1966 (2225.8 kg ha⁻¹), and Suba (1534.8 kg ha⁻¹). In agreement with our results, a multi-environment field study (Wosene et al., 2015) also indicated that Ethiopian barley landraces or farmer varieties show higher yield stability and comparable yield to improved varieties. A similar study conducted in Ethiopia (Lakew et al., 1997), Syria (Ceccarelli, 1996) and Spain (Yahiaoui et al., 2014) also supported our study and concluded that landraces outperformed improved varieties grown in favorable to modern stress conditions.

3.3 PAIRWISE CORRELATION ANALYSIS

Results of the pairwise Spearman rank correlation coefficient (r_s) among 10 morpho-agronomic traits are displayed in Figure 3. The estimates of trait association revealed that a strong positive association exists among pheno-agronomic traits such as DTH-DTM ($r_s = 0.9$), DTM-GFP ($r_s = 0.67$), and GY-PH ($r_s = 0.5$). In contrast, a strong negative correlation was observed between KNS and TKM ($r_s = -0.62$). However, most of the trait associations showed weak correlations

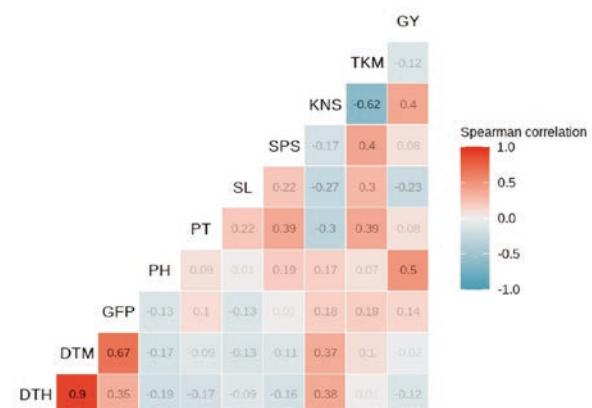


Figure 3: Spearman correlations of 10 morpho-agronomic traits of barley germplasm accessions. Keys: no correlation ($-0.1 \leq r_s \leq 0.1$), weak correlation ($-0.3 \leq r_s < -0.1$ or $0.1 < r_s \leq 0.3$), moderate correlation ($-0.3 < r_s < -0.5$ or $0.3 < r_s < 0.5$) and strong correlation ($r_s < -0.5$ or $r_s > 0.5$) where r_s is Spearman correlation. Trait abbreviations are given in Table 1 footnote.

Table 2: Mean, minimum, and maximum values of agro-morphological parameters of Ethiopian barley genotype and local released varieties based on regional diversity

Region	Morpho- agronomic traits [†]									
	DTH	DTM	GFP	PH	PT	SL	SPS	KNS	TKM	GY
Amhara (n = 153)	Mean	72.9	113.7	40.8	86.7	1.7	8.2	20.5	35.0	48.7
	Min	54	89	31	57.8	0.2	5.2	14.1	14.3	31.9
	Max	97	141	59	108.4	3.7	11.2	50.5	52.2	3599.9
Oromia (n = 41)	Mean	73.4	114.8	41.4	85.1	1.8	8.2	19.9	35.0	48.2
	Min	59	96	31	67.6	0.6	5.4	16.0	18.8	31.9
	Max	99	146	55	99.7	3.0	11.5	39.9	49.0	3511.9
Tigray (n = 32)	Mean	70.0	111.0	41.0	82.1	2.1	8.1	21.5	26.6	52.4
	Min	58	101	31	69.2	0.8	5.9	16.8	18.2	45.6
	Max	82	126	47	100.6	3.5	10.4	32.0	53.9	3055.3
Local checks (n = 3)	Mean	79.8	127	47	76.8	1.9	8.4	20.3	38.2	49.6
	Min	79	124	45	73.6	1.4	7.8	16.8	24.9	46.1
	Max	81	132	51	81.8	2.6	9.5	26.2	46.6	51.6
Accessions (n = 226)	Mean	72.6	113.5	40.9	85.7	1.8	8.2	20.6	33.8	49.1
	Min	54	89	31	57.8	0.2	5.2	14.1	14.3	31.9
	Max	99	146	59	108.4	3.7	11.5	50.5	53.9	3599.9
Genotypes (n = 229)	Mean	72.7	113.7	41.0	85.6	1.8	8.2	20.5	33.9	49.1
	Min	54	89	31	57.8	0.2	5.2	14.1	14.3	31.9
	Max	99	146	59	108.4	3.7	11.5	50.5	53.9	3599.9

Min-minimum, Max-maximum; [†]Trait abbreviations are given in Table 1 footnote

(44 %) followed by no correlations (29 %), moderate correlations (18 %) and strong correlations (9 %). For instance, moderate correlation was found for KNS-GY ($r_s = 0.4$), TKM-SPS ($r_s = 0.4$), TKM-SL ($r_s = 0.3$), TKM-PT ($r_s = 0.39$), DTM-KNS ($r_s = 0.37$), DTH-KNS ($r_s = 0.38$), DTH-GFP ($r_s = 0.35$) traits. Similarly, GY showed a weak correlation with all phenological traits. The better the correlations among traits the more likely it is that breeders can indirectly select one trait based on other traits with high heritability. From the breeders point of view, the strong correlation between GY and PH ($r_s = 0.5$) is found to be problematic with respect to lodging and yield penalty. Therefore, reducing plant height without incurring grain yield is highly recommended to escape lodging as also reported by Monteagudo et al. (2019) study that found a positive correlation between PH and GY.

3.4 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) was estimated to elucidate the spatial distribution and diversity of genotypes and eventually identify the key traits that contributed utmost to the observed variations. In this study, the principal component analysis based on the correlation between 10 measured traits was used to discern the pattern of variation for the studied 229 barley genotypes (Table 3). The first four principal components (PCs) having Eigenvalue ≥ 1 and explained 74.3 % of the total cumulative variance present in the studied barley germplasm were retained (Table 3, Figure S1 a & b). Likewise, Yadav et al., (2018) reported Eigenvalue ≥ 1 that explained a total variability of 79.5 %. In addition, (Bedasa et al., 2015) and (Demissie & Bjørnstad, 1996) reported four principal components explaining a total variance of 72 % and 63 % in the studied 102 and 49 barley accessions respectively.

Table 3: Eigen values, Eigen vectors, variance, cumulative variance and contribution of variables to four dimensions obtained from the considered 10 morpho-agronomic traits

Traits [†]	PC1	PC2	PC3	PC4
DTH	-0.50 (24.99)	0.19 (3.80)	0.15 (2.15)	-0.26 (6.81)
DTM	-0.55 (29.74)	0.28 (8.06)	0.05 (0.22)	-0.14 (1.92)
GFP	-0.37 (13.36)	0.31 (9.80)	-0.16 (2.68)	0.16 (2.54)
PH	0.09 (0.86)	-0.12 (1.35)	-0.57 (32.69)	-0.39 (15.58)
PT	0.20 (4.12)	0.38 (14.4)	-0.25 (6.36)	0.09 (0.85)
SL	0.22 (5.05)	0.28 (7.89)	0.09 (0.88)	-0.66 (43.64)
SPS	-0.10 (1.08)	0.23 (5.36)	-0.35 (12.2)	0.48 (23.52)
KNS	-0.42 (17.65)	-0.37 (13.7)	-0.16 (2.52)	-0.19 (3.43)
TKM	0.16 (2.52)	0.55 (30.58)	-0.18 (3.41)	-0.10 (0.99)
GY	-0.08 (0.64)	-0.22 (5.05)	-0.61 (36.88)	-0.09 (0.73)
Eigenvalue	2.7	2.0	1.8	1.0
Variance (%)	27.0	19.8	17.7	9.8
Cumulative variance (%)	27.0	46.9	64.6	74.3

Values in bold indicate the highest absolute values among all four PCs for each trait which represents relative contribution and values in parentheses indicate factor loadings with > 10 % positive contribution to the variation. [†]Trait abbreviations are given in Table 1 footnote.

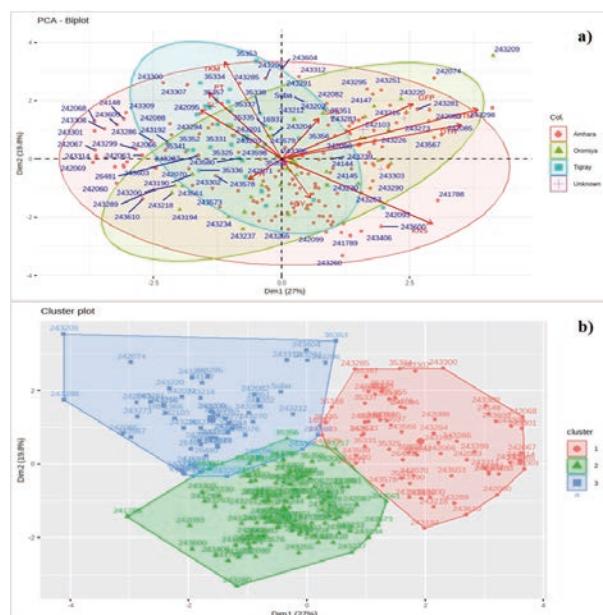


Figure 4: A PCA biplot analysis based on the mean value of 10 morpho-agronomic traits depicting the scatter of 226 barley accessions and three checks along with their measured traits and entire collection sites (a) and a Principal Component Analysis (PCA) biplot where the samples are colored by cluster (b). Trait abbreviations are given in Table 1. The vector indicates the traits and unknown represents improved varieties (Suba, HB-1307, and HB-1966). The more the deviation from the center towards the direction of the vector, the higher the value and vice versa. In other words, an angle of 90° or less between any two vectors (traits) indicates a positive correlation and vice versa.

Factor loadings were considered with > 10 % positive contribution (Figure S1c & d, Table 3) and indicated that DTH (24.99), DTM (29.74), KNS (17.65), and GFP (13.36) were mostly contributed for PC1 in the negative direction. The major traits that contributed to PC2 were TKM (30.58) in the positive direction and PT (14.4) and KNS (13.7) in the negative direction. Similarly, GY (36.88), PH (32.69), and SPS (12.2) were contributed for PC3 in the negative direction while SL (43.64) and PH (15.58) attributed for PC4 in the negative direction and SPS (23.52) in the positive direction. In line with our study (Dido et al., 2020) reported that DTH was heavily loaded in the negative direction for PC1. In contrast, the findings of (Fantahun et al., 2023a) indicated that PC1 was mostly affected by phenological traits in the positive direction while DTM was highly loaded.

To visualize the associations of genotypes and measured traits, a PCA scatter biplot was generated based on the first two PCs (Figure 4a). The first and the second PCA biplot accounted for 46.8 % of the total variability among the genotypes, indicating that DTH, DTM, GFP, TKM, KNS, and PT were considered as the most discriminating traits contributing to the highest variability (Figure 4a, Table 3). The genotypes positioned on the top right quadrant were characterized by late heading and maturity whereas, the genotypes clustered on the bottom right quadrant were associated with the highest GY and KNS; the genotypes positioned on the top left quadrant are associated with high TKM, PT and SL whereas, the genotypes assembled at the bottom left quadrant is char-

acterized by high PH, early heading and maturity. On the other hand, the genotypes concentrated around the origin had similar genetic characteristics, while the genotypes that were found far from the origin are considered as unrelated genotypes (Figure 4a). Therefore, the distribution of the genotype over the four quadrants indicates the presence of significant genetic diversity among the studied barley genotypes. On the other hand, a PCA cluster biplot (Figure 4b) indicated that 229 barley genotypes were grouped in three major clusters.

3.5 CLUSTERING ANALYSIS

Clustering is grouping of individuals according to their similarities. Cluster analysis (Figure 5) based on 10 quantitative traits for 229 barley genotypes were grouped into three major clusters, each with a trait-specific pattern. The mean values of the three clusters for the 10 quantitative traits of the tested barley genotypes (Table 4) showed that a high cluster mean was recorded in cluster (I) for PH, KNS, and GY and the lowest for PT and TKM. These findings suggest that the genotypes in cluster I could be preferable for improving grain yield while maintaining high plant height, making them suitable for both animal fodder and human consumption. cluster (II) exhibited the lowest mean value for all phenological traits studied and the highest value for PH, PT, SL, and TKM. This indicates that the genotypes in cluster II could serve as a valuable genetic resource for developing early maturing and high-yielding barley cultivars, particularly suited for short rainy seasons. In contrast, the genotypes in cluster III were characterized by late heading and maturity, a high number of spikes per plant (SPS), and shorter plant height compared to the other clusters. These traits make cluster III genotypes a potential source to cross-hybridize and develop dwarf barley varieties suitable for extended rainy seasons.

Among the 229 genotypes studied, 97 (42.4 %)

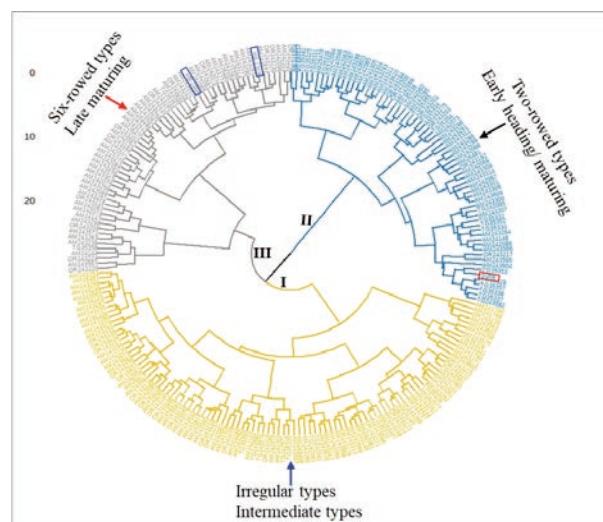


Figure 5: A circular dendrogram-based phylogenetic tree of 229 barley genotypes obtained from 10 quantitative traits. Genotypes highlighted in the blue box (cluster III) indicate six-rowed improved barley genotypes and red box (cluster II) for two-rowed ones. The prefix presented before each accession number indicates their region of collection (e.g. AM-Amhara, OR-Oromia, TG-Tigray).

were grouped in Cluster I, which exhibited intermediate phenological characteristics. Meanwhile, 71 genotypes (31 %) were classified as early-maturing (cluster II), and 61 genotypes (26.6 %) were late-maturing (cluster III) (Table 4). Statistical tests further confirmed that the observed differences among clusters were significant for DTH, DTM, GFP, PH, SL, SPS, KNS, and GY. However, no significant differences were found for TKM and PT (Table 4). Overall, the genotypes were clustered based on morphological similarity rather than their collection region. This is consistent with previous studies (Bedasa et al., 2015; Benlioglu et al., 2025; Kaur et al., 2022), which found that cluster analysis groups genotypes according to morphological traits and less on their geographic location. Figure 5 further illustrates that two-

Table 4: Cluster means and size among three clusters generated from 10 morpho-agronomic traits

Clusters	†Traits										Cluster size
	DTH	DTM	GFP	PH	PT	SL	SPS	KNS	TKM	GY	
I	72.4	112.0	39.5	85.6	1.51	7.9	19.9	41.3	45.3	2398.0	97 (42.4%)
II	64.1	102.8	38.7	86.9	2.19	8.6	21.0	22.6	52.5	1893.5	71 (31%)
III	83.0	129.1	46.1	83.6	1.74	8.2	21.7	35.1	51.4	1929.3	61 (26.6%)
<i>p</i> -values	0.027	0.008	0.049	0.000	0.211	0.036	0.007	0.000	0.632	0.000	

The means/medians of the clusters are significantly different for the traits if $p < 0.01$ (highly significant), $p < 0.05$ (significant), and $p > 0.05$ (non-significant); †Trait abbreviations are found in Table 1 footnote

Table 5: Intra (diagonal and bold) and inter (above diagonal) cluster distance (D2) among three clusters generated from 229 barley genotypes

Clusters	I	II	III
I	3.83	4.51	4.57
II		4.08	5.06
III			3.69

rowed barley accessions were predominantly grouped in cluster II, six-rowed ones in cluster III, and a mix of irregular and six-rowed barley spikes in cluster I. Similar findings were reported by (Fantahun et al., 2023a).

The Euclidean distance analysis revealed the highest genetic distance (10.02) between accession numbers AM-242060 (early heading/maturing) and OR-243209 (late heading/maturing). In contrast, the smallest genetic distance (0.68) was observed between accessions TG-25357 and TG-35329, both collected from the Tigray region, indicating their similar genetic backgrounds (Additional File 1, Sheet 3). These results align with the findings of Angassa and Mohammed (2021), who reported a genetic distance range of 0.61 to 8.06 among 138 barley genotypes. The inter-cluster distances based on the Euclidean dissimilarity matrix (Table 5) showed the highest distance (5.06) between clusters II and III, suggesting that these clusters are genetically distinct and makes them ideal candidates for crossing to create high-yielding, early-maturing, or dwarf barley varieties with diverse traits. In contrast, the lowest (4.51) inter-

cluster distance between clusters I and II revealed that the clusters are genetically similar or less divergence in traits. On the other hand, the highest intra-cluster distance (4.08) observed in cluster II suggests greater genetic diversity or variability among the genotypes within the cluster. In contrast, the low intra-cluster distance (3.69) in cluster III indicates that the genotypes within a cluster are genetically similar or closely related. Overall, the highest and lowest values of intra-cluster and inter-cluster distances provide important insights into the genetic diversity and relationships among the clusters and genotypes.

3.6 ESTIMATES OF VARIANCE COMPONENTS, HERITABILITY, AND GENETIC ADVANCE

The genetic variability (Table 6) was computed to estimate the level of genetic variation for environmental and genetic factors. High magnitude of phenotypic variance ($\sigma^2 p$) and genotypic variance ($\sigma^2 g$) were observed in GY, DTM, PH, KNS and DTH and low magnitude in SL, PT, SPS, TKM and GFP traits. Because of variations in mean and measurement units, the value of phenotypic and genotypic variants cannot be directly compared across traits (Gadissa et al., 2021). Therefore, coefficient of variation at phenotype and genotype levels was used to compare variability among traits (Table 6). The coefficient of variation study indicated that the highest PCV and GCV were noted on GY (39.18 and 35.08), PT (38.41 and 31.19), KNS (29.56 and 29.03) and SPS (28.83 and 28.44) indicating high degree of genetic variability and

Table 6: Genetic variability analysis of 10 yield and yield-related traits of 229 barley genotypes

Trait	Mean \pm SE	Range	$\sigma^2 p$	$\sigma^2 g$	$\sigma^2 e$	GCV (%)	PCV (%)	ECV	$h^2 b$ (%)	GA	GAM (%)
DTH	72.70 \pm 0.62	54-99	92.43	88.07	4.37	12.92	13.23	2.88	95.28	18.90	26.01
DTM	113.7 \pm 0.79	89-146	143.71	125.80	17.90	9.87	10.55	3.72	87.54	21.65	19.04
GFP	41.0 \pm 0.33	31-59	20.52	7.87	12.65	6.84	11.04	8.67	38.34	3.58	8.73
PH	85.6 \pm 0.56	57.8-108.4	102.22	80.42	21.80	10.47	11.81	5.45	78.67	16.41	19.16
PT	1.78 \pm 0.04	0.2-3.7	0.47	0.31	0.16	31.19	38.41	22.42	65.95	0.93	52.26
SL	8.2 \pm 0.07	5.2-11.5	1.31	0.98	0.33	12.07	13.97	7.03	74.68	1.76	21.52
SPS	20.7 \pm 0.4	12.5-33.9	35.60	34.63	0.96	28.44	28.83	4.75	97.29	11.98	57.87
KNS	33.9 \pm 0.66	14.3-53.9	100.27	96.71	3.56	29.03	29.56	5.57	96.45	19.92	58.82
TKM	49.1 \pm 0.34	31.9-62.4	26.39	24.48	1.91	10.07	10.45	2.81	92.78	9.83	20.01
GY	2118.3 \pm 43.3	292.3-3599.9	688720.7	552309.5	136411.1	35.08	39.18	17.44	80.19	1372.97	64.82

Keys: $\sigma^2 p$ -phenotypic variance, $\sigma^2 g$ -genotypic variance, $\sigma^2 e$ -environmental variance, GCV-genotypic coefficient of variance, PCV-phenotypic coefficient of variance, ECV-environmental coefficient of variance, $h^2 b$ -heritability in the broad sense, GA-genetic advance, GAM-genetic advance over mean. PCV, GCV and GAM values < 10 % (low), 10-20 % (medium), > 20 % (high); $h^2 b$ values < 30 (low), 30-60 (medium), and \geq 60 (high). Trait abbreviations are given in Table 1 footnote.

close relationship with yield attributing traits and thus could be a useful trait for screening yield traits. Whereas medium PCV and GCV values were observed on the remaining traits except for DTM and GFP which showed low GCV. The magnitude of PCV value was generally greater than GCV for all studied traits as expected and revealed that the apparent variation was due to the environmental factors up to some extent. However, the magnitude of the difference was low for all studied traits suggesting the observed variation was due to genetic factors and less influenced by environmental factors. Moreover, the low GCV estimates for DTM and GFP suggest a direct selection on these traits for improvement depending on phenotype expression. In line with our findings, high coefficient of variation (PCV and GCV) were reported by (Addisu & Shumet, 2015; Angassa, 2021; Zewodu et al., 2025) in barley for SPS, KNS and GY traits.

Broad sense heritability (h^2) of a trait represents the extent at which the genotypes are affected by environment and environmental errors (You et al., 2017). High heritability was obtained on SPS (97.29 %) followed by KNS (96.45 %), DTH (95.28 %), TKM (92.78 %), DTM (87.54 %), GY (80.19 %), PH (78.67 %), SL (74.68 %) and PT (65.95 %) while low for GFP (38.34 %) (Table 6). Similar results have been reported by Addisu and Shumet (2015) and Zewodu et al. (2025). Estimation of heritability coupled with genetic advance offers the most suitable condition for selection (Singh and Chaudhary, 1985). High heritability and genetic advance were observed for GY, KNS, SPS, PT, DTH, SL and TKM traits (Table 6) which revealed the presence of additive genetic variance in the trait (Johnson et al., 1955) and suggested that reliable crop improvement could be effective through phenotypic selection. Similar studies in barley (Angassa, 2021; Kaur et al., 2022; Kumar & Shekhawat, 2013; Shrimali et al., 2017) and flax (Abeje & Zewodu, 2024; You et al., 2017) also indicated that estimates of heritability with genetic advance are more reliable than individual consideration of the parameters for selection.

4 CONCLUSION

Understanding the extent and magnitude of phenotypic diversity and trait associations is essential for the maximum utilization of gene bank materials in breeding programs and conservation. This study examined the phenotypic diversity of 226 Ethiopian barley landraces and three improved varieties using variance components and multivariate analysis. Significant genetic variability was found in most traits, except for GFP, which had non-significance differences and medium heritability. Key traits such as grain yield (GY), days to

maturity (DTM), and plant height (PH) showed notable variability, aiding breeders in selection efforts. PCA biplot analysis identified early (Acc-243314, 242070 and 242060) and late heading/maturing types (Acc-243210, 243209, 243273, 243298 and 243074). In agreement with PCA Biplot results, cluster analysis grouped genotypes into three major clusters according to their morphological similarity and rarely on their geographical location. Notably, Accession 241790 collected from the Amhara region exhibited low GFP, low PH, and high GY (above the grand mean of $2118.3 \text{ kg ha}^{-1}$) and rarely have composite spike forms. The study suggests further multi-location trials to explore genes linked to composite spikes and other valuable traits. In conclusion, the superior grain yield of landraces compared to improved varieties highlights the potential of Ethiopian farmer varieties for breeding programs aimed at enhancing food security.

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6 CONFLICT OF INTEREST

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 SUPPLEMENTARY MATERIALS

The following list of supplementary tables and figures are attached with this manuscript.

Additional File 1 (Xlsx): Geospatial data of the studied barley accessions (sheet 1; Passport), Mean performance of 229 barley genotypes based on 10 morpho-agronomic traits (Sheet 2; Mean performance) and Euclidean distance between the studied barley genotypes (Sheet 3; Genetic distance)

Figure S1: A scree plot of four dimensions/PCs (a&b) and contribution of variables (c&d) retained from 10 morpho-agronomic traits

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9 CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Gizie Abeje: Conceptualization, Visualization, Validation, Methodology, Investigation, Data curation, Formal analysis, Resources, Project administration, Supervision, Funding acquisition, Writing—original draft, Writing-review, and editing.

Alemayehu Zewodu: Resources, Visualization, Supervision, Investigation, Data curation, Validation, Writing-review, and editing.

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