

Research article

Assessment of Phenotypic Diversity and Trait Relationships in Barley Genotypes Using Genotype × Trait Biplot Analysis

Naser Sabaghnia¹, Hamid Ghorbanian¹, Asghar Ebadi-Segherloo² and Mohsen Janmohammadi^{1*}

¹University of Maragheh, Department of Plant Production and Genetics, Faculty of Agriculture, Maragheh, Iran

²Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, 1136756199, Ardabil, Iran

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Abstract

Barley is an important cereal crop with extensive genetic variation, which is essential for sustainable crop improvement and food security. This investigation evaluated the genetic diversity among twenty barley genotypes through morphological traits using a genotype × trait biplot approach. A field trial was conducted in the Moghan district and fertile tillers (FT), tillers per plant (TP), seeds per plant (SP), single-plant yield (YSP), seed yield performance (SYP), thousand-seed weight (TSW), plant height (PH), straw yield (SY), spike length (SL), and biological yield (BY) were measured. The biplot explained 66% of the variation (43% and 23% by the first and second components, respectively), effectively capturing genotype-trait interaction. Vector analysis revealed positive associations among yield-related traits, with SP correlated YSP, SYP associated with TSW, and SY, PH, SL, and BY forming a correlated group. Tiller-related traits were independent of biomass-related traits, indicating that selection for higher tillers may improve specific yield components without affecting plant size or straw yield. The polygon-view biplot identified vertex genotypes exhibiting trait-specific superiority; G10 excelled in SP, YSP, SYP, and TSW; G12 was superior in FT and TP; and G13 performed best for SY, PH, SL, and BY. Genotypes such as G5, G7, and G16 were located in less favorable sectors, indicating suboptimal performance. The ideal trait view highlighted SYP as the most informativeness trait, followed by YSP, TSW, and SP, suggesting that these traits are most valuable for multi-trait selection. The biplot approach visualized phenotypic diversity and trait relationships and identified promising barley genotypes for selection.

Keywords: genotype evaluation; genetic improvement; seed yield; yield components

*Corresponding author: E-mail: mjanmohammadi@maragheh.ac.ir

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1. Introduction

Plant genetic resources constitute the fundamental foundation of agricultural development and simultaneously function as a safeguard against environmental fluctuations. The depletion and erosion of these valuable resources remains a threat to world food security and long-term sustainable agricultural systems (Khatri et al., 2024). Despite their importance, modern breeding practices and the narrowing of phenotypic diversity and trait relationships have led to the underutilization of available genetic resources, thereby limiting yield improvement and adaptive potential in crops. The conservation and effective utilization of plant genetic resources, particularly indigenous and locally adapted populations, have therefore become essential components of future breeding strategies (Salgotra & Chauhan, 2023). The accelerating loss of phenotypic diversity and trait relationships, coupled with the increasing demand for resilient and high-performing cultivars, has placed plant genetic resources at the forefront of global scientific and agricultural concern. Selection is one of the principal objectives of plant breeding and represents a highly effective approach for identifying and exploiting phenotypic diversity as well as understanding relationships among agronomic traits (Swarup et al., 2021). Through selection, breeders are able to identify genetically superior individuals and incorporate favorable traits into breeding programs. Plant breeders rely on existing genetic variation either for direct utilization or for transferring desirable genes into improved populations (Singer et al., 2021). The long-term success of breeding efforts is thus inherently dependent on the conservation of genetic reserves. Despite the extensive use of multivariate analyses in barley breeding, there is limited information on the application of genotype $\times$ trait (GT) biplot analysis to evaluate morphological and yield-related traits in barley genotypes adapted to the semi-arid conditions of the Moghan region. This knowledge gap limits the ability to identify high-performing genotypes for multi-trait selection in this specific agroecological context.

Numerous studies have demonstrated the existence of substantial phenotypic diversity and trait relationships within global germplasm collections of barley, particularly in regions such as Iran, which is recognized as one of the primary centers of origin for several important crop species (Hosseini et al., 2025; Shahmoradi, 2026). Within the Middle East, the Fertile Crescent is considered one of the most diverse regions for barley, attracting considerable attention from breeders due to its high levels of genetic variability and endemism. The direct or indirect incorporation of indigenous traits of barley from this region into crossbreeding programs has proven to be of great importance (Chaurasia & Bhatia, 2025). Multivariate analysis of Egyptian barley genotypes indicated considerable variation and interrelationships among agronomic traits, whereas yield was most related with the number of spikes per square meter and grains per spike, which also exerted the greatest direct and indirect effects on yield, highlighting them as key targets for selection in barley breeding programs (Abdelghany et al., 2025). Evaluation of barley genotypes using morphological traits indicated pronounced phenotypic diversity, with highly significant differences observed across all traits, whereas yield was associated with key characteristics such as tillers per plant, spike length, seeds per spike, plant height, and thousand-grain weight, indicating their usefulness as indirect selection criteria for effective selection in barley breeding programs (Aka et al., 2025). Evaluation of Ethiopian barley genotypes demonstrated high variation across all agronomic traits, whereas yield exhibited wide variability and showed strong positive phenotypic and genotypic associations with key traits, particularly spikelets per spike, fertile tillers, thousand-seed weight, and seeds per spike, indicating their effectiveness as selection criteria, and highlighting their potential value for barley improvement and genetic conservation (Zewodu et al., 2025).

Evaluation of barley genotypes based on morphological traits showed high genetic variability and clear associations among yield-related characteristics, whereas yield indicated relationships with grains per spike, grain weight per spike, and thousand-seed weight. On the other hand, test weight was negatively associated with heading date, while the genotype $\times$ trait biplot effectively differentiated the genotypes and identified high-yielding individuals, demonstrating the usefulness of multivariate approaches for selecting superior barley genotypes in breeding programs (Güngör et al., 2024). Evaluation of barley genotypes under stress conditions indicated significant genotype-dependent variation in agronomical performance, particularly seed yield, whereas high variation was observed in plant growth and yield-related traits, indicating the presence of exploitable morphological variability for selection under contrasting environmental conditions (Bouhraoua et al., 2025). Despite the availability of numerous studies applying multivariate and biplot approaches in barley, limited information is available for locally adapted germplasm under the semi-arid conditions of the Moghan region. The novelty of the present study lies in (i) the evaluation of diverse barley genotypes under these specific agro-climatic conditions, (ii) the identification of trait association patterns, including the observed partial independence between tillering and biomass-related traits within this dataset, and (iii) the integrated application of multiple genotype $\times$ trait biplot views to support multi-trait selection. These aspects provide useful insights for region-specific barley improvement strategies, phenotypic diversity and trait relationships.

2. Materials and Methods

To assess phenotypic diversity and trait relationships, twenty barley genotypes were evaluated in a field experiment conducted during the 2023-2024 growing season at the Moghan Research Station using a randomized complete block design with three replications. The experimental site was located in the Moghan region (northwestern Iran), which was characterized by a semi-arid climate with warm summers, an average annual temperature of approximately 15 °C, and mean annual precipitation of about 335 mm. The soil at the site was clay loam with neutral pH, and was of moderate salinity, and low organic matter content. The plant materials consisted of twenty barley genotypes, including advanced breeding lines and locally adapted germplasm developed by regional breeding programs. The genotypes represented diverse genetic backgrounds and were selected to capture variation in agronomic performance. Most genotypes were of the spring growth type. One commercial cultivar, Mahour, was included as a check to provide a benchmark for comparison under the experimental conditions. Detailed information on genotype origin and characteristics is presented in Table 1, which shows phenotypic diversity and trait relationships. Each experimental plot consisted of six rows, each 3.0 m in length, with a plant spacing of 10 cm within rows and 30 cm between rows. Prior to sowing, the field was deeply ploughed and disked to create a uniform seedbed, and seeds were manually sown in mid-November. All plots received uniform agronomic management throughout the growing season. Fertilizers were applied at recommended rates, including 100 kg ha⁻¹ nitrogen (N), 60 kg ha⁻¹ phosphorus (P₂O₅), and 40 kg ha⁻¹ potassium (K₂O). Phosphorus and potassium were applied as basal doses prior to sowing, while nitrogen was split into two applications: half at sowing and the remaining half at the tillering stage. The experiment was conducted under supplemental irrigation conditions, with irrigation applied at key growth stages, including tillering, stem elongation, and grain filling, depending on rainfall conditions. Weed control was performed manually and, when necessary, supplemented with selective herbicides. Pest and disease management practices were applied based on

field monitoring to minimize biotic stress and ensure optimal crop growth. For trait measurement, ten plants were randomly selected from the central rows of each plot to avoid border effects. Plants were selected using a systematic random sampling approach along the rows to ensure informativeness sampling. Fertile tillers (FT), as number of productive tillers bearing spikes per plant (plant basis, count), tillers per plant (TP), as number of tillers per plant (plant basis, count), seeds per plant (SP), as number of seeds produced per plant (plant basis, count), and single-plant yield (YSP), as grain weight per individual plant (g plant^{-1}), were recorded. Also, plant height (PH), was measured from the soil surface to the tip of the spike (excluding awns) at maturity (cm), and spike length (SL), was quantified as length of the main spike excluding awns (cm). Straw yield (SY) was recorded as dry weight of vegetative biomass per plot after grain removal (kg plot^{-1}), while biological yield (BY), was measured as aboveground biomass (grain plus straw) per plot (kg plot^{-1}). Seed yield performance (SYP), was quantified as grain yield per plot, adjusted to a standard moisture content of 12% (kg plot^{-1}). Finally, thousand-seed weight (TSW), was recorded as weight of 1000 seeds measured from three random samples per plot (g). At physiological maturity, plants from the central rows were harvested to determine plot-based traits (SY, BY, and SYP), while plant-based traits were recorded from the sampled individuals. All yield-related measurements were standardized to a uniform moisture content (12%) to ensure comparability.

Table 1. Twenty barley genotypes including nineteen advanced lines with a check cultivar

#	Name or Pedigree
G1	Mahour
G2	CBSS97M00850T-G-2M-1Y-2M-0Y-02
G3	CB06M00193T-C-9M-0AP-0AP-0AP
G4	CBSS07Y00696S-0AP-0AP
G5	CBSS05M00256S-2M-0Y-0M-0AP-0TR
G6	CBSS97M00850T-G-2M-1Y-2M-0Y-06
G7	CBSS06Y00079S-29Y-0M-0AP-0TR
G8	ICB86-0512-1AP-0TR-4AP-0TR-0AP
G9	ICB05-0498-5AP-0AP
G10	ICB05-0421-0AP-6AP-0AP
G11	ICB95-0204-0AP-16AP-0AP-4AP-0AP-9AP-0AP
G12	LBIRAN/UNA80//LIGNEE640/6/Vmorales
G13	CBSS05Y00158S- 25Y-0M-0Y-0M-4AP
G14	CBSW01WM00107T-0TOPY-6Y-1M-2Y-1M-0Y
G15	CBSS97M00850T-G-2M-1Y-2M-0Y-13
G16	ICB08-0124- OAP - OG-OG-OG- 5G
G17	CBSS05M00680D-K-2M-0M-0AP-0TR
G18	ICB09-1436-0AP-0TR-0AP-0TR-0AP-0TR
G19	TR145/I95039
G20	M91178002/Mahigan

The distribution of each trait was examined for normality using the Anderson-Darling test, which evaluates whether the data significantly depart from a normal distribution pattern via Minitab 17.0 (Minitab Ins., USA). Also, Levene's test was applied via SPSS 25.0 (IBM-SPSS Ins., USA) to verify the equality of variances across genotypes, ensuring that differences in variability would not bias the results of next analyses. The level of significance for above statistical tests was set at $P \leq 0.05$. Thus, the dataset was confirmed to satisfy the required assumptions, which ensured the validity and robustness of subsequent parametric analyses.

A genotype $\times$ trait interaction framework was applied to investigate the relationships among barley genotypes and their evaluated traits. The analysis was performed via the GGEbiplot software (Yan, 2001), which allows simultaneous visualization of genotypes and traits in a single biplot, providing a visual representation of genotypes and multiple traits simultaneously. Unlike principal component analysis (PCA) or cluster analysis, which summarize variation or group genotypes without showing trait-specific performance, the GT biplot allows identification of genotypes that excel in particular traits, visualization of trait correlations, and assessment of multi-trait performance, making it particularly suitable for informed selection decisions in barley breeding programs. The model used for constructing the biplot is expressed as:

$$\frac{a_{ij} - b_j}{S_j} = \sum_{n=1}^2 \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij}$$

where a_{ij} is the value of genotype i for characteristic j , b_j is the mean of genotypes for characteristic j , S_j is the square root of the variance for characteristic j , λ_n is the eigen value for component n , ξ_{in} and η_{jn} are loadings for genotype i and characteristic j on component n , respectively, and ε_{ij} is the error magnitude, which is not explained by the components. Prior to GT biplot analysis, trait data were standardized (mean-centered and scaled by standard deviation) to remove scale effects. The biplot was constructed based on singular value decomposition (SVD) of the standardized data matrix, with the first two principal components (PC1 and PC2) retained for graphical visualization. Symmetrical scaling was applied to allow simultaneous interpretation of genotypes and traits. Interpretation of the biplot followed established principles, where vector length indicates informativeness ability, angles between vectors approximate correlations, and polygon and ideal-genotype views are used to identify superior genotypes. This approach allows the decomposition of the variation into components, enabling identification of the important traits contributing to variability among genotypes and facilitating the selection of superior genotypes. By plotting genotypes and traits in a graph, breeders can visually assess trait associations, detect patterns of genotype performance, and prioritize genotypes for breeding programs.

3. Results and Discussion

The first two principal components explained 66% of the total variation, indicating that a portion of the variability was not captured in the biplot. Therefore, the graphical interpretations should be considered approximate rather than definitive. In addition, as the experiment was conducted in a single environment, the observed relationships and genotype performance should be interpreted as environment specific. Further multi-environment evaluations are required to confirm the multi trait performance in this environment and general applicability of these findings. This proportion of explained

variance indicated that the biplot effectively captured the majority of the information in the dataset, providing acceptable informativeness regarding trait relationships and genotypic differences, suggesting that this model can meaningfully summarize complex patterns and revealed correlations among traits (Triana-Martinez et al., 2025). Vectors originating from the graph's origin indicated trait directions, with the cosine of the angles covering the informativeness of their association; cosine $0^\circ = +1$ as positive relation, and cosine $90^\circ = 0$ as no relation. Elongated vectors suggested that traits contributed significantly to the identified variability. The important associations included positive correlations within this dataset between fertile tillers (FT), and tillers per plant (TP); between seeds per plant (SP), and yield of single plant (YSP); between seed yield performance (SYP), and thousand seed weight (TSW); and among plant height (PH), straw yield (SY), spike length (SL), and biological yield (BY), regarding the close angles of their vectors (Figure 1). Aka et al. (2025) reported positive relations between tillers per plant and seed yield or yield-related traits. Moreover, tillers per plant, spike length, plant height, and thousand-seed weight correlated positively with yield performance, indicating their usefulness as selection traits in breeding programs. Furthermore, Abebe (2024) found positive associations between seeds per plant, seeds per spike, yield components and yield performance in other barley germplasms.

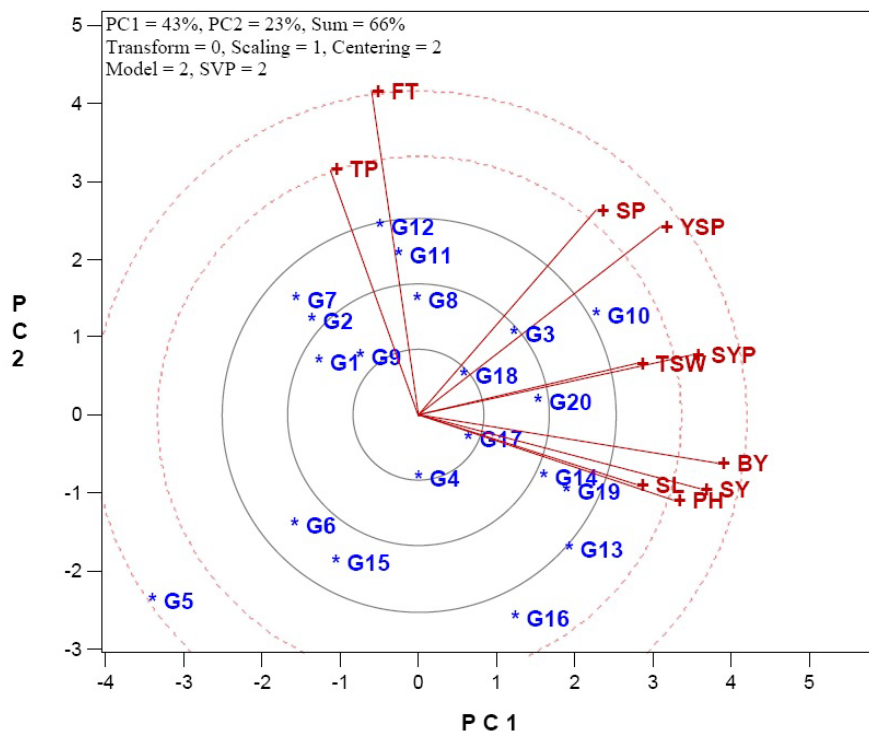


Figure 1. Vector-view facility of genotype × trait biplot analysis in barley genotypes

Tiller related traits such as FT and TP within this dataset showed near-zero associations with SY, PH, SL, and BY, as indicated by nearly perpendicular vectors within this dataset (Figure 1). Thus, in these barley materials, the tillers contributed independently

to yield components rather than being tightly correlated with plant biomass or architecture. Previous studies reported positive correlations among biomass-related traits and yield components (Shamuyarira et al., 2023; Shirvani et al., 2025). However, the relationship between tiller number and biomass traits can vary depending on genotype and environmental conditions. Some studies observed weak or non-significant correlations between tiller number and plant height or biomass (Kassie & Tesfaye, 2019; Bogale & Percze, 2025), suggesting that these traits were genetically and physiologically regulated by largely independent mechanisms. These observations were consistent with the near-zero associations observed in the current investigation, highlighting that selection for higher tillers may improve certain yield components without necessarily affecting plant size or straw yield under the conditions of this study. The identified biplot associations reflected the overall data structure and traits relationships rather than exact pairwise associations such as Pearson coefficients. Furthermore, the majority of the identified trait relationships were in good agreement with those indicated by the numerical correlation matrix because the genotype $\times$ trait biplot generally captured these associations, providing a visual confirmation of the correlations; however, some inconsistencies were apparent. This discrepancy likely arose because the model explained only 66% of the observed variation, meaning that a portion of trait variability remained noninformativeness (Sabaghnia et al., 2024), and consequently, not all associations could be perfectly reflected.

The polygon-view genotype $\times$ trait biplot facilitated comparison among barley genotypes, highlighting those that performed best in specific traits (Figure 2). The hexagon enclosed vertex genotypes allowed identification of ideal barley genotypes, with G10 excelling in seeds per plant (SP), yield of single plant (YSP), seed yield performance (SYP), and thousand seed weight (TSW). Genotype G12 performed best in tillering including fertile tillers (FT), and tillers per plant (TP), while genotype G13 was the best in plant height (PH), straw yield (SY), spike length (SL), and biological yield (BY). Other remaining vertex genotypes, including G5, G7, and G16, occupied less favorable sectors (Figure 2), indicating suboptimal performance in any of measured barley traits. Thus, the genotype $\times$ trait biplot provided a structural overview rather than precise mean values but effectively distinguished higher performance under the conditions of this study from inferior genotypes.

Güngör et al. (2024) demonstrated the effectiveness of genotype $\times$ trait biplot analysis for identifying higher performing genotypes and visualizing trait relationships in barley, lending support to the patterns observed in current barley study. In barley germplasm evaluated for morphological traits, the fitted biplot model accounted for over 60% of variation and successfully identified high-performing genotypes for yield-related traits (Güngör et al., 2024). Vertex genotypes corresponded to higher performance under the study conditions whereas those positioned near the origin were considered average or less favorable, a pattern similar to the positioning of current investigation that revealed trait-specific superiority.

The ideal trait facility of the genotype $\times$ trait biplot (Figure 3) evaluated traits based on their informativeness potential. Vector length indicated the capability of informativeness, while the proximity of a trait's projection to the mean coordinate reflected its relative contribution. However, seed yield performance (SYP) showed the highest capability for both informativeness and contribution, followed by yield of single plant (YSP), thousand seed weight (TSW), and seeds per plant (SP). Traits such as fertile tillers (FT), and tillers per plant (TP), displayed good informativeness potential but limited contribution. However, the informativeness capability of plant height (PH), straw yield (SY), spike length (SL), and biological yield (BY), were moderate (Figure 3).

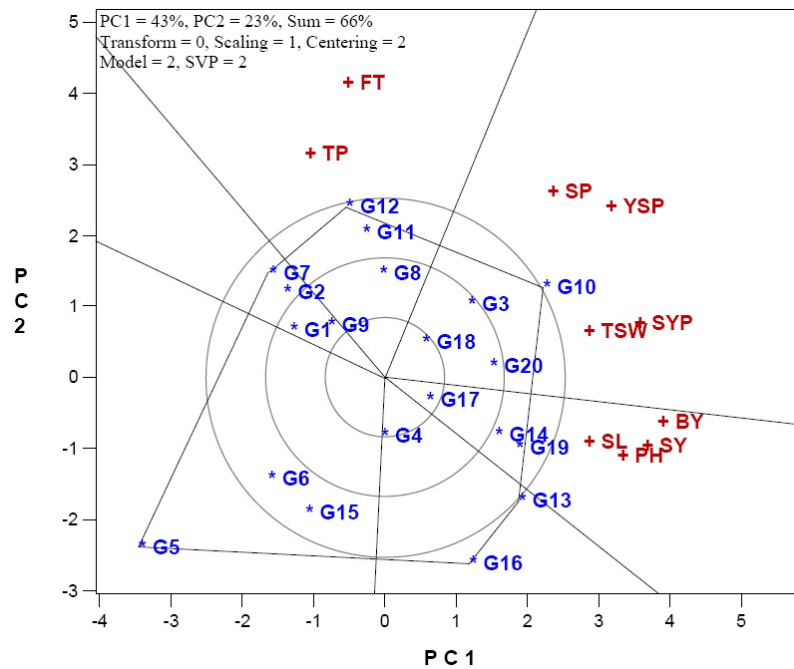


Figure 2. The polygon-view facility of genotype × trait biplot analysis in barley genotypes

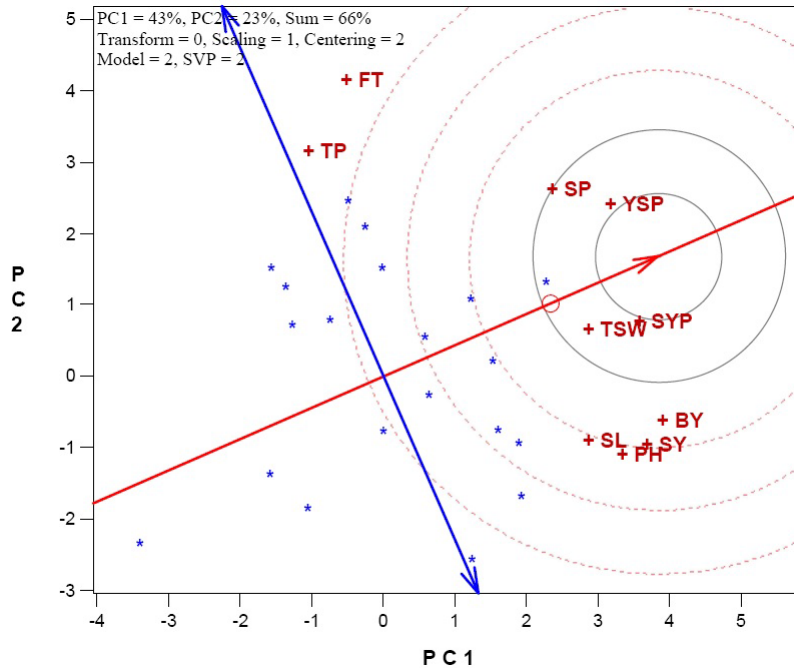


Figure 3. The ideal-trait facility of genotype × trait biplot analysis in barley genotypes

The assessment of trait informativeness trait contribution and informativeness ability provides valuable guidance for barley breeding. Traits with both high informativeness and informativeness trait contribution (yield related traits) were particularly informative for selecting higher performance (Shirzad et al., 2025), as they captured major variation and effectively differentiated among genotypes. Traits with strong informativeness but limited informativeness trait contribution (tillering capability) were useful for distinguishing genotypes in specific aspects but may not fully reflect performance. In contrast, traits with moderate informativeness and informativeness trait contribution, (SY, PH, SL, and BY), provided complementary information but were less decisive for selection. However, they showed smaller vector lengths, lower contributions to principal components, or had weaker genotype discrimination in a genotype $\times$ trait (GT) biplot, so they provided less information for separating genotypes compared with highly informative traits. Finally, identifying traits that combine high informativeness trait contribution, and informativeness power allows breeders to focus on key traits that simultaneously capture variation and effectively distinguish higher performance (Gholizadeh & Ghaffari, 2023), thereby improving the efficiency of multi-trait selection strategies.

Figure 4 highlighted the best genotype regarding most traits as a reference point, and genotypes closest to this hypothetical position—G10, followed by G3, G20, and G18—emerged as the most effective ones. Other genotypes, G5, followed by G6, and G15, showed inferior performance. Furthermore, five genotypes (G1, G2, G4, G7, and G9), similar to the three mentioned unfavorable genotypes, were located below the average axis and were considered as unfavorable ones.

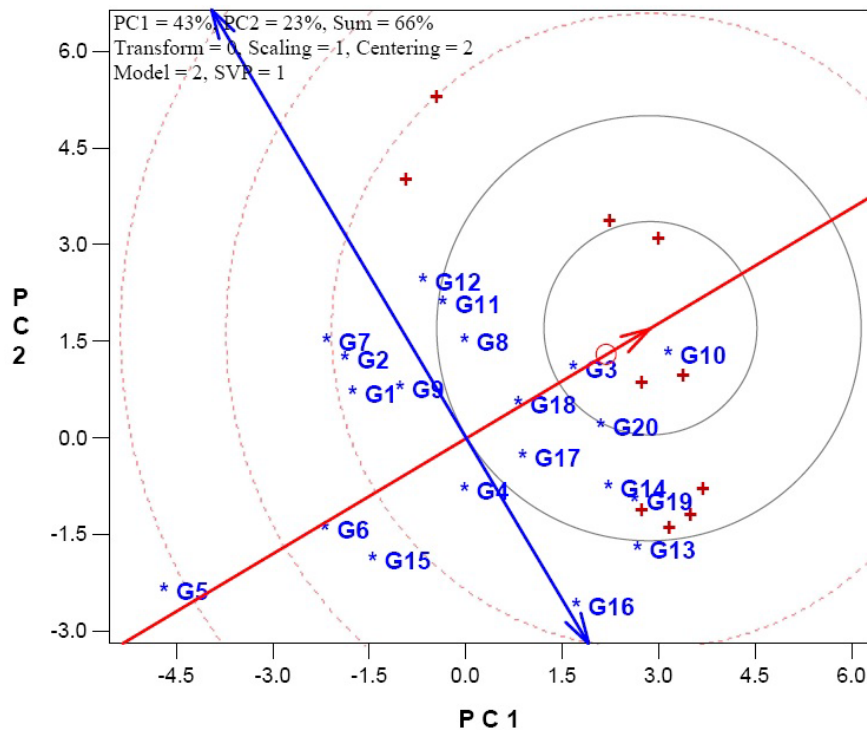


Figure 4. The ideal-genotype facility of genotype $\times$ trait biplot analysis in barley genotypes

Genotype G1 was the check cultivar (Mahour), which was not included in the capability assessment, while the twelve above average genotypes (3, 8, 10-14, and 16-20), indicated more power for distinguishing barley traits. This facility of genotype $\times$ trait biplot provided a practical framework for identifying higher performance under the conditions of this study of barley genotypes across multiple traits (Karahan & Akgün, 2020). Genotypes positioned closest to the ideal reference demonstrated desirable trait values and exhibited high performance, making them promising candidates for selection in breeding programs. Genotypes located below the average axis showed limited performance and were less suitable for multi-trait improvement. By integrating multiple traits into a single reference point, this approach facilitated informed selection decisions, enabling breeders to prioritize genotypes that balance high productivity, stability, and trait complementarity (Nur et al., 2024).

Analysis of seed yield performance (SYP) across twenty barley genotypes indicated that genotype G10 following to G3, G13, G14, G19, and G20, exceeded high performance (Figure 5). Also, genotype G5 followed by G6 and G15 indicated the lowest seed yield performance. Regarding the average axis of SYP, the performance of SYP was higher than average in genotypes 3, 8, 10-14, and 16-20, while the performance of SYP was lower than average in genotypes G1, G2, G4, G5, G6, G7, G9, and G15 (Figure 5). The performance of twelve barley genotypes was better than check cultivar Mahour, thus the potential of these lines was higher than existing cultivars.

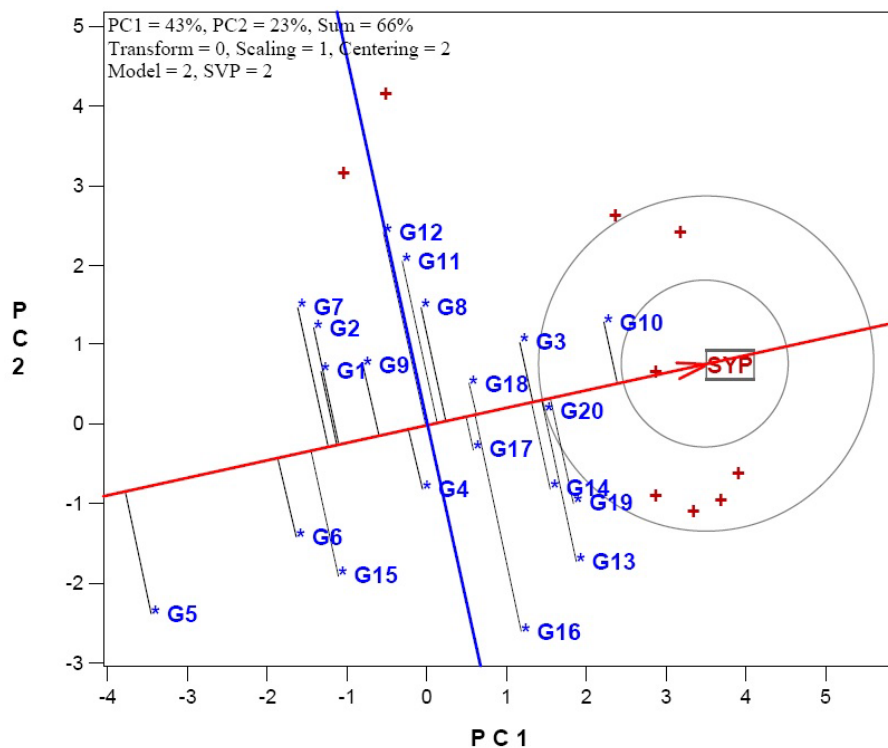


Figure 5. Ranking of barley genotypes based on the seed yield performance (SYP), in the genotype $\times$ trait biplot analysis

The genotype $\times$ trait biplot analysis in the current investigations provided a framework for understanding the relationships among morphological and yield-related traits in barley, as well as for identifying higher performance under the conditions of this genotype study for multi-trait selection, which is important for breeding strategies in barley germplasm. The observed grouping of certain traits, for example, seeds per plant, yield per single plant, seed yield performance, and thousand-seed weight, highlighted the close integration of yield-determining factors in barley. These traits, which exhibited both high informativeness trait contribution and informativeness potential can be considered primary targets for selection in breeding programs. Similar patterns were reported in previous studies on barley germplasm. Yang et al. (2025) found that seed yield components like seeds per spike and per plant were strongly related with yield performance across diverse barley genotypes, supporting the relevance of these traits as indicators of productivity. Also, Hussein and ALshugeairy (2022) observed positive correlations between tiller number, spike-related traits, and yield in barley genotypes, suggesting that multi-trait selection emphasized yield components could be effective for improving overall performance.

Tiller-related traits, including fertile and total tillers, exhibited near-zero associations with biomass-related traits like straw yield, plant height, spike length, and biological yield. This decoupling suggested that tiller formation in these genotypes was largely independent of plant architecture and biomass accumulation. Previous studies reported similar observations, whereas Teklemariam et al. (2022) found weak correlations between tiller number and biomass traits in Ethiopian barley, indicating that genetic regulation of tillering may differ from that of plant height or biomass accumulation. Mmbando (2025) also highlighted that the contribution of tiller number to yield components varied depending on environmental conditions, which may explain the variation observed in this study. Such independence implies that selection for increased tillering could enhance yield components without necessarily increasing vegetative biomass, providing breeders with flexibility in targeting specific yield traits. The correlation among biomass-related traits, like plant height, spike length, straw yield, and biological yield, is consistent with previous reports in barley, like that of Stolarski et al. (2022), who demonstrated that plant height and straw yield varied simultaneously and contributed to biomass production but may not directly correlated with reproductive output. This may have implications for yield components and resource allocation, although further multi-environment studies are required to confirm these relationships.

The polygon and ideal-genotype facilities allowed the identification of genotypes that were best for specific traits or provided superior balance of multiple desirable attributes. Genotype G10 demonstrated higher performance under the conditions of this study for yield-related traits, whereas genotypes G12 and G13 performed well in tillering and biomass-related traits, respectively. These findings aligned with previous genotype $\times$ trait biplot studies in barley by GÜngör et al. (2024), who showed that vertex genotypes in a biplot framework effectively captured higher performance under the conditions of this study performance in targeted traits. Moreover, genotypes positioned near the origin often reflected average or below-average performance. Similarly, Kumar et al. (2025) emphasized the utility of multi-trait biplots in highlighting genotypes that showed a balance high productivity, stability, and complementary trait performance. The differentiation between genotypes based on specific traits underscored the importance of multi-trait selection in barley. While some genotypes performed well in yield components, they may not be optimal for biomass accumulation or tillering capacity. Genotypes with high tillering but moderate yield components may be suitable in harsh environments where compensatory growth improves resilience, but genotypes with high seed yield and

moderate vegetative growth may be preferred for high-input systems focused on seed production. This highlights the potential of the biplot approach to inform context-specific selection strategies rather than relying solely on univariate measures (Pour-Aboughadareh et al., 2025).

Assessing the informativeness trait contribution and informativeness ability of traits provided crucial guidance for selection priorities. In current investigation, yield-related traits exhibited both high informativeness trait contribution and informativeness, whereas tiller-related traits showed high informativeness but limited informativeness trait contribution. These findings were consistent with previous reports on barley breeding. Shirzad et al. (2025) emphasized that traits with high informativeness and informativeness trait contribution were informative for selecting genotypes that captured major variation while effectively differentiating among genotypes. Fauzan et al. (2025) also highlighted that focusing on traits with both high informativeness trait contribution and informativeness enhanced the efficiency of multi-trait selection programs, particularly when resources limited extensive field evaluation. However, this apparent decoupling should be interpreted cautiously, as it reflects patterns observed under the specific conditions of this study and may vary across environments and genetic backgrounds. This approach offers an advantage over traditional selection methods that may overemphasize yield alone. By integrating traits with strong informativeness and informativeness potential, breeders can identify genotypes that combine high yield with desirable morphological or physiological characteristics, increasing the likelihood of selecting higher performance under the conditions of this study lines with stable performance across environments.

The applying of genotype $\times$ trait biplot analysis into barley breeding programs offers several practical advantages. First, it provides a visual framework for simultaneously evaluating multiple traits, reducing reliance on single-trait selection and enabling informed decisions that consider trait complementarity. Second, it identifies both genotypes with broad superiority across multiple traits and those that excel in specific traits, allowing breeders to tailor selection strategies to the target environment or production system. Third, the assessment of trait informativeness trait contribution and informativeness aids in prioritizing traits that are both informative and efficient for selection, optimizing resource allocation in breeding trials. The differentiation of genotypes observed in this study also has implications for pre-breeding and hybridization strategies. Genotypes exhibiting complementary strengths, like high yield combined with strong tillering or biomass production, can be used as parents in crosses to combine desirable attributes, enhancing genetic gain. Also, the identification of genotypes that outperform the check cultivar Mahour suggested that the evaluated germplasm harbors valuable variation for improving yield and associated traits in local barley breeding programs.

Finally, the current study demonstrated that genotype $\times$ trait biplot analysis is a powerful tool for evaluating barley germplasm, capturing major trait variation, and informing multi-trait selection. Yield-related traits like seeds per plant, yield per single plant, seed yield performance, and thousand-seed weight were highly informative for selection, while tillering traits contributed complementary information without strong associations with biomass. The identification of genotypes with high multi-trait performance, as well as those excelling in specific traits, provided a practical framework for breeding strategies aimed at improving barley productivity. The study identified high-potential genotypes, including G10, which excelled in yield-related traits, and G12, which showed superior performance in tillering. These genotypes represent valuable resources for barley breeding programs aimed at improving productivity and supporting sustainable agricultural practices for food security. It should be noted that the first two principal components explained 66% of the total variation, indicating that a portion of the variability was not captured in the biplot.

Therefore, the graphical interpretations should be considered approximate rather than definitive. In addition, the experiment was conducted in a single environment, which limits the generalization of the results. Multi-environment trials are thus recommended to validate the stability and adaptability of the superior genotypes identified under the study conditions.

4. Conclusions

The present study demonstrated that genotype $\times$ trait biplot analysis is a useful approach for evaluating trait relationships and genotype performance in barley under semi-arid conditions. Yield-related traits, including seeds per plant, single-plant yield, seed yield performance, and thousand-seed weight, showed strong associations and exhibited high informativeness and informativeness ability, indicating their importance as selection criteria. Tiller-related traits appeared largely independent of biomass-related traits within this dataset, suggesting distinct patterns of trait association under the experimental conditions. Genotype G10 showed higher performance for yield-related traits, while G12 and G13 were associated with tillering and biomass-related traits, respectively. Several genotypes also outperformed the check cultivar, indicating their potential for further evaluation. However, the findings of this study are subject to several limitations. The experiment was conducted in a single location and a single growing season, and only morphological traits were considered. In addition, the number of evaluated genotypes was limited. Therefore, the results should be considered preliminary, and further multi-environment trials are required to validate genotype performance, stability, and adaptability before any recommendation for cultivar release or large-scale use can be made.

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6. Authors' Contributions

M.J., and A.E.: designed research
H.GH., and A.E.: performed research
N.S., A.E., and M.J.: contributed new reagents/analytic tools
N.S., and A.E.: analyzed data
H.GH., and A.E.: coordinated research
M.J., and N.S.: wrote the paper

ORCID

Naser Sabaghnia  <https://orcid.org/0000-0001-9690-6525>

Asghar Ebadi-Segherloo  <https://orcid.org/0000-0001-7132-7762>

Hamid Ghorbanian  <https://orcid.org/0009-0001-2469-6167>

Mohsen Janmohammadi  <https://orcid.org/0000-0002-6121-6791>

7. Conflicts of Interest

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

References

- Abdelghany, M., Saad, K. I., Dakrory, M., & Amer, K. E. (2025). Multivariate analysis of agronomic characteristics in some Egyptian barley landraces: a field-based quantitative study. *BMC Plant Biology*, 25(1), Article 1565. <https://doi.org/10.1186/s12870-025-07512-8>
- Abebe, K. (2024). Cluster and principal component analysis for yield and yield related traits of food barley (*Hordeum vulgare* L.) genotypes at Woreilu district, South Wollo, Ethiopia. *Agricultural and Biological Research*, 40(3), 1086-1090.
- Aka, S. R., Sarker, U., Talukder, M. Z. A., Azam, M. G., Ercisli, S., Hossain, N., Alharbi, M. S., & Ullah, R. (2025). Genetic variation of barley genotypes using morphological traits, amylose content, and molecular markers. *Scientific Reports*, 15(1), Article 34840. <https://doi.org/10.1038/s41598-025-19242-w>
- Bogale, A. A., & Percze, A. (2025). The influence of primary soil tillage methods and foliar nutrient provision on the growth, yield, and associated traits of winter barley (*Hordeum vulgare* L.). *Acta Agraria Debreceniensis*, 1, 19-26. <https://doi.org/10.34101/actaagrar/1/15452>
- Bouhraoua, S., Ferioun, M., Boussakouran, A., Belahcen, D., Hammani, K., & Louahlia, S. (2025). Comprehensive screening and evaluation of diverse Mediterranean barley genotypes for salt tolerance using multiple agro-morphological and tolerance indices criteria. *Reproduction and Breeding*, 5(4), 171-179. <https://doi.org/10.1016/j.repbre.2025.09.002>
- Chaurasia, S., & Bhatia, S. (2025). Use of wild relatives in breeding programs to develop climate resilient wheat. In M. K. Khan, A. Pandey, M. Hamurcu, & S. Gezgün (Eds.). *Wheat wild relatives* (pp. 361-399). Academic Press. <https://doi.org/10.1016/B978-0-443-22090-6.00013-8>
- Fauzan, M. R., Abdullah, M. A., Ridara, F., Ustari, D., & Ruswandi, D. (2025). Dataset of multi-trait analysis of maize hybrid under shading and drought stress based on GGE biplot, MTSI and MDIGI. *Data in Brief*, 63, Article 112148. <https://doi.org/10.1016/j.dib.2025.112148>
- Gholizadeh, A., & Ghaffari, M. (2023). Genotype by yield* trait (GYT) biplot analysis: A novel approach for phenotyping sunflower single cross hybrids based on multiple traits. *Food Science and Nutrition*, 11(10), 5928-5937. <https://doi.org/10.1002/fsn3.3524>
- Güngör, H., Türkoğlu, A., Çakır, M. F., Dumlupınar, Z., Piekutowska, M., Wojciechowski, T., & Niedbała, G. (2024). GT biplot and cluster analysis of barley (*Hordeum vulgare* L.) germplasm from various geographical regions based on agro-morphological traits. *Agronomy*, 14(10), Article 2188. <https://doi.org/10.3390/agronomy14102188>
- Hosseini, M., Mojab, M., Yassaie, M., Zand, E., Keshtkar, E., Revolinski, S., VanWallendael, A., & Mamnoie, E. (2025). Wild barley (*Hordeum vulgare* L. subsp. *Spontaneum* (C. Koch) Thell.): a noxious weed species in Iran and an untapped genetic resource for barley cultivar development-molecular approach. *Acta Physiologiae Plantarum*, 47(10), Article 92. <https://doi.org/10.1007/s11738-025-03839-9>
- Hussein, N. J., & Alshugeairy, Z. K. K. (2022). Effect genetic correlation of growth characteristics and yield of barley under different seeding rates. *Journal of Kerbala for Agricultural Sciences*, 9(4), 171-182. <https://doi.org/10.59658/jkas.v9i4.1072>

- Karahan, T., & Akgün, I. (2020). Selection of barley (*Hordeum vulgare*) genotypes by GYT (genotype x yield x trait) biplot technique and its comparison with GT (genotype x trait). *Applied Ecology and Environmental Research*, 18(1), 1347-1359.
- Kassie, M., & Tesfaye, K. (2019). Influence of variety and nitrogen fertilizer on productivity and trait association of malting barley. *Journal of Plant Nutrition*, 42(10), 1254-1267. <https://doi.org/10.1080/01904167.2019.1605380>
- Khatri, P., Kumar, P., Shakya, K. S., Kirlas, M. C., & Tiwari, K. K. (2024). Understanding the intertwined nature of rising multiple risks in modern agriculture and food system. *Environment, Development and Sustainability*, 26(9), 24107-24150. <https://doi.org/10.1007/s10668-023-03638-7>
- Kumar, R., Kumar, M., Gangwar, L. K., Kumar, V., Singh, S., Edhigalla, P., & Rahimi, M. (2025). Association and reliability analysis of multi-trait selection methods and selection of superior genotypes across the traits in Indian mustard. *Scientific Reports*, 15(1), Article 23405. <https://doi.org/10.1038/s41598-025-07721-z>
- Mmbando, G. S. (2025). Tiller number: an essential criterion for developing high-yield and stress-resilient cereal crops. *Archives of Agronomy and Soil Science*, 71(1), 1-21. <https://doi.org/10.1080/03650340.2025.2464662>
- Nur, A. A., Sugiharto, A. N., Soegianto, A., Nafisah, N., & Wahyudi, M. I. (2024). Genotype-by-trait (GT) biplot analysis and ranking of rice genotypes in salinity stress conditions. *Acta Agrobotanica*, 77(1), 1-15. <https://doi.org/10.5586/aa/190216>
- Pour-Aboughadareh, A., Jadidi, O., Jamshidi, B., Bocianowski, J., & Niemann, J. (2025). Dataset for unveiling the application of multi-trait genotype-ideotype distance index and multi-trait index based on factor analysis and ideotype-design models in the identification of high-yielding and stable barley genotypes. *Data in Brief*, 59(1), Article 111383. <https://doi.org/10.1016/j.dib.2025.111383>
- Sabaghnia, N., Zakeri, M., Karimizadeh, R., & Janmohammadi, M. (2024). Entry by tester biplot model for evaluation of some kabuli chickpea genotypes based on several multiple traits. *Agriculture and Forestry*, 70(2), 25-36.
- Salgotra, R. K., & Chauhan, B. S. (2023). Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*, 14(1), Article 174. <https://doi.org/10.3390/genes14010174>
- Shahmoradi, S. (2026). Multivariate analysis of genetic diversity in two-row and six-row barley (*Hordeum vulgare* L. ssp. *vulgare*) germplasm in national plant Genebank of Iran. *Genetic Resources and Crop Evolution*, 73(1), Article 16. <https://doi.org/10.1007/s10722-025-02689-1>
- Shamuyarira, K. W., Shimelis, H., Figlan, S., & Chaplot, V. (2023). Combining ability analysis of yield and biomass allocation related traits in newly developed wheat populations. *Scientific Reports*, 13(1), Article 11832. <https://doi.org/10.1038/s41598-023-38961-6>
- Shirvani, H., Mehrabi, A. A., Farshadfar, M., Safari, H., Arminian, A., & Fatehi, F. (2025). Genetic structure and marker-trait association analysis for agro-morphological and physiological characteristics in germplasm of wild barley (*Hordeum vulgare* subsp. *spontaneum*). *Scientific Reports*, 15(1), Article 38706. <https://doi.org/10.1038/s41598-025-22401-8>
- Shirzad, A., Asghari, A., Moharramnejad, S., Shiri, M., & Ebadi, A. (2025). Integrated analysis of genotype by yield trait and genotype by environment interactions for selecting superior maize genotypes. *Scientific Reports*, 15(1), Article 41372. <https://doi.org/10.1038/s41598-025-25254-3>
- Singer, S. D., Laurie, J. D., Bilichak, A., Kumar, S., & Singh, J. (2021). Genetic variation and unintended risk in the context of old and new breeding techniques. *Critical Reviews in Plant Sciences*, 40(1), 68-108. <https://doi.org/10.1080/07352689.2021.1883826>
- Stolarski, M. J., Krzyżaniak, M., & Olba-Zięty, E. (2022). Biomass yield and quality of perennial herbaceous crops in a double harvest in a continental environment. *Industrial Crops and Products*, 180, Article 114752. <https://doi.org/10.1016/j.indcrop.2022.114752>

-
- Swarup, S., Cargill, E. J., Crosby, K., Flagel, L., Kniskern, J., & Glenn, K. C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science*, 61(2), 839-852. <https://doi.org/10.1002/csc2.20377>
- Teklemariam, S. S., Bayissa, K. N., Matros, A., Pillen, K., Ordon, F., & Wehner, G. (2022). The genetic diversity of Ethiopian barley genotypes in relation to their geographical origin. *PLoS One*, 17(5), Article e0260422. <https://doi.org/10.1371/journal.pone.0260422>
- Triana-Martinez, J., Álvarez-Meza, A., & Castellanos-Dominguez, G. (2025). Enhancing agricultural data interpretability and visualization with Tabnet-driven feature extraction and local biplots. *Results in Engineering*, 27(1), Article 106672. <https://doi.org/10.1016/j.rineng.2025.106672>
- Yan, W. (2001). GGEbiplot-A windows application for graphical analysis of multi-environment trial data and other types of two-way data. *Agronomy Journal*, 93(5), 1111-1118. <https://doi.org/10.2134/agronj2001.9351111x>
- Yang, J., Ahmed, H. G. M. D., Akram, M. I., Iqbal, R., Alwahibi, M. S., Elshikh, M. S., & Zeng, Y. (2025). Drought-induced trait correlations in barley genotypes through a multi-trait selection approach. *Applied Ecology and Environmental Research*, 23(3), 5657-5674.
- Zewodu, A., Mohammed, W., & Shiferaw, E. (2025). Genetic variability and association of morpho-agronomic traits among Ethiopian barley (*Hordeum vulgare* L.) accessions. *Scientifica*, 2025, Article 3957883. <https://doi.org/10.1155/sci5/3957883>