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Exploring Phenotypic Diversity and Yield Attributes in Six-Row Barley (*Hordeum vulgare* L.) Genotypes

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ABSTRACT The present study aimed to explore phenotypic diversity and yield-related traits in ten six-row barley (*Hordeum vulgare* L.) genotypes (G1–G10) under normal field conditions. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications, and data were recorded on seven key agronomic traits: plant height (PH), flag leaf area (FLA), spikelets per spike (SPS), spike length (SL), number of grains per spike (NGS), 100-grain weight (HGW), and grain yield per plant (GYP). ANOVA revealed highly significant (p < 0.01) differences among genotypes for PH (MS = 16.62), FLA (MS = 5.84), SPS (MS = 3.41), SL (MS = 1.57), NGS (MS = 30.76), and GYP (MS = 13.45), indicating considerable genetic variation. Descriptive statistics showed PH ranged from 88.9 to 96.0 cm, FLA from 24.5 to 29.4 cm², SPS from 18.8 to 22.1, and GYP from 30.6 to 36.3 g. Genotype G8 consistently outperformed others across traits, followed by G5 and G3. Correlation analysis revealed strong positive associations between GYP and NGS (r = 0.94**), SPS (r = 0.91**), PH (r = 0.89**), and FLA (r = 0.88**), highlighting their relevance in indirect selection. HGW and SL showed weak or non-significant correlations with GYP. The study concludes that traits such as NGS, SPS, FLA, and PH are reliable yield indicators and should be prioritized in breeding programs targeting high-performing barley cultivars.

Keywords: Phenotyping; Yield; Genotypes; Variability; Correlation; Selection

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INTRODUCTION Barley (*Hordeum vulgare* L.) is one of the most ancient and widely cultivated cereal crops, ranking fourth globally in terms of production after maize, rice, and wheat. It has historically played a vital role in the development of agriculture and human civilization, particularly in arid and semi-arid regions where it thrives under challenging environmental conditions (Ahmed et al., 2025, Zewodu et al., 2025). As a crop of significant agro-ecological and economic importance, barley is used for diverse purposes, including food, feed, malt, and industrial applications. In recent years, barley has gained renewed attention for its nutritional properties, such as high dietary fiber, betaglucan content, and the presence of essential bioactive compounds, making it a candidate for health-promoting functional foods (Akdogan et al., 2025, Yirgu et al., 2022).

Barley's adaptability to a wide range of environments—including saline, drought-prone, and low-fertility soils—makes it a crucial crop in marginal areas where other cereals fail to thrive. This adaptability is underpinned by a vast genetic pool that enables the species to survive and produce yield under various climatic and edaphic stresses. However, climate change and growing population pressure are intensifying the need to develop barley cultivars that are not only high yielding but also resilient to biotic and abiotic stresses. This necessitates the identification and selection of genotypes that possess superior agronomic traits and stable performance across diverse environments (Amezrou et al., 2018, Watt, 2020).

Genetic variability is the foundation for any crop improvement program. The success of plant breeding lies in the availability and utilization of heritable variation present in the

germplasm pool. In barley, morphological and yield-related traits such as plant height, flag leaf area, spike characteristics, grain weight, and grain yield per plant are important selection criteria. These traits are often polygenic and exhibit quantitative inheritance, meaning their expression is influenced by both genetic and environmental factors. Therefore, assessing the extent of variation among genotypes for these traits is essential to identify promising lines for future breeding programs (Bardehji et al., 2024, Verma et al., 2021).

Plant height is a critical trait associated with lodging resistance, biomass accumulation, and overall plant vigor. Flag leaf area contributes significantly to the photosynthetic capacity during grain filling, directly impacting yield. Spike-related traits, including spikelets per spike and spike length, determine the number of grains that can be produced per unit spike, thereby influencing final grain yield. The number of grains per spike and 100-grain weight are direct yield components and reflect the genotype's capacity for grain production and resource utilization efficiency. Grain yield per plant is a comprehensive trait affected by multiple physiological and morphological parameters, and it serves as a key performance indicator in field evaluations (Barral Baron, 2016; Tondelli et al., 2013).

Correlation analysis is another critical component of plant breeding research. It provides information about the degree and direction of association among various traits, which is invaluable for indirect selection strategies. For instance, a strong positive correlation between flag leaf area and grain yield suggests that improving leaf area can lead to higher yields. Conversely, a negative correlation between plant height and yield might indicate the need to balance height for stability without compromising productivity. Understanding these relationships helps breeders prioritize traits during selection and develop ideotypes with optimized trait combinations for specific environments (Belay and Kebede, 2025, Rossi, 2023).

In Pakistan, barley is grown mainly in rainfed and low-input systems, often on marginal lands. Despite its adaptability, the national average yield remains below its potential due to the cultivation of outdated, low-performing varieties and suboptimal agronomic practices. To enhance productivity and meet the growing demand for food and feed, there is an urgent need to identify and promote high-yielding, well-adapted genotypes. Evaluating the performance of available germplasm under local conditions is a fundamental step toward achieving this goal (Benlioglu et al., 2025, Rahal-Bouziane et al., 2015).

The current study was undertaken to evaluate the morphological and yield-related traits of ten barley genotypes under normal growing conditions using RCBD with three replications. The genotypes were selected based on their diverse genetic backgrounds and potential for high yield and stress resilience (Benlioglu et al., 2025, Kumar et al., 2020). The primary objectives were to assess the extent of genetic variability among the genotypes for key agronomic traits, determine the significance of observed differences through analysis of variance (ANOVA), and explore trait interrelationships through correlation analysis. This approach allows for the identification of promising genotypes and trait combinations that could be incorporated into

future breeding programs aimed at improving barley productivity and adaptability (Bensemane et al., 2011, Kaur et al., 2019).

MATERIALS AND METHODS

The present study was conducted to evaluate the morphological and yield-related performance of ten barley (Hordeum vulgare L.) genotypes, designated as G1 to G10. These genotypes were selected based on their distinct agronomic characteristics and potential for yield enhancement. The experiment was carried out using a Randomized Complete Block Design (RCBD) with three replications to ensure the reliability of the data and to account for environmental variability across blocks. Each genotype was sown in a plot comprising three rows of 2 meters in length, with a row-to-row spacing of 25 cm and plant-to-plant spacing of 10 cm. Uniform agronomic practices, including irrigation, fertilizer application, weeding, and pest control, were followed throughout the growing season to ensure optimum growth conditions across all treatments.

Data were recorded on seven key morphological and yield-related traits. These included plant height (cm), measured from the soil surface to the tip of the spike excluding awns; flag leaf area (cm²), calculated using the formula length × width × 0.75; spikelets per spike, counted manually on the main tiller; spike length (cm), measured from the base to the tip of the spike; number of grains per spike, determined after threshing the main spike; 100-grain weight (g), measured by weighing a random sample of 100 seeds using a digital analytical balance; and grain yield per plant (g), calculated by harvesting, threshing, and weighing the grains from each selected plant. For each replication, five representative plants were randomly selected per plot for data collection to ensure statistical reliability.

The recorded data were subjected to Analysis of Variance (ANOVA) to determine the presence of significant differences among genotypes for each trait. The ANOVA was performed based on the RCBD model, and the mean square values were used to calculate F-values. The significance of genotypic effects was evaluated at both 5% and 1% probability levels, and the error mean squares were used to assess experimental precision. Additionally, Pearson's correlation coefficients were computed among the studied traits to understand the degree and direction of relationships between morphological and yield components. These correlations provided insight into the potential indirect selection for yield improvement through associated traits. All statistical analyses were performed using standard software packages such as R (version 4.2.1), ensuring robust and accurate interpretation of results.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) presented in Table 1 provides critical insights into the variability among ten barley genotypes for morphological and yield-related traits under normal growing conditions. The results revealed highly significant differences (p < 0.01) among genotypes for most traits, including plant height, flag leaf area, spikelets per spike, spike length, number of grains per spike, and grain yield per plant, while 100-grain weight

showed non-significant variation (Bernád et al., 2024, Kaur et al., 2019).

The significant genotypic differences for plant height (MS = 16.62), flag leaf area (MS = 5.84), and spike-related traits suggest that these characteristics are under strong genetic control, providing a solid basis for selection and genetic improvement. The high mean square values for number of grains per spike (30.76) and grain yield per plant (13.45) indicate substantial genotypic influence, highlighting the potential of certain genotypes to outperform others in grain production. This variability is essential for breeding programs targeting yield enhancement, as it reflects the presence of exploitable genetic diversity within the evaluated germplasm (Bretani, 2019, Jan et al., 2022).

In contrast, the non-significant differences observed among replications for all traits indicate uniform experimental conditions across blocks, affirming the reliability of the RCBD layout. Similarly, the non-significance of 100-grain weight (MS = 0.43) among genotypes may suggest either low genetic variability for this trait or a strong environmental influence, which diminishes its utility as a primary selection criterion under the current testing conditions (Desta et al., 2024; Hemshrot et al., 2019). Overall, the ANOVA results emphasize that genotypic variation is a key determinant of performance in most traits, particularly yield and its contributing factors, thereby providing a solid foundation for selecting superior genotypes in barley improvement programs (Desta et al., 2024; Dickin et al., 2012).

Descriptive analysis

The descriptive analysis of the evaluated traits in ten 6-row barley genotypes revealed considerable phenotypic variability, indicating substantial scope for selection and genetic improvement (Table 2). Plant height (PH) ranged from 88.9 cm (G7) to 96.0 cm (G8), with a mean of 92.41 cm, standard deviation (SD) of 2.31, and a coefficient of variation (CV) of 2.5%. The moderate range and low CV indicate a relatively stable trait with limited environmental influence. Similar ranges of variation in PH were reported by (Dido et al., 2020) suggesting that plant stature in barley is moderately heritable and influenced by both genetics and agronomic conditions. Taller genotypes like G8 may provide better biomass, but excessively tall plants may increase lodging risk under high-input conditions (Bardehji et al., 2024, Hemshrot et al., 2019).

Flag leaf area (FLA) showed values from 24.5 cm² (G7) to 29.4 cm² (G8) with a mean of 26.82 cm², SD of 1.39, and a CV of 5.19%, indicating moderate variability. Leaf area is directly related to photosynthetic capacity and assimilate supply to the developing grain. The findings align with those of [Kumar et al., 2019], who emphasized the importance of flag leaf area in determining sink-source relationships and yield potential under both normal and stress conditions. The moderate variability in FLA suggests that selection for larger leaf area could contribute to yield improvement (Bensemane et al., 2011, Kumar et al., 2020).

Spikelets per spike (SPS) ranged from 18.8 (G7) to 22.1 (G8), averaging 20.47, with an SD of 1.02 and CV of 4.97%. This trait exhibited noticeable genotypic variation, consistent with the

findings of (Jan et al., 2022, Rossi, 2023), who reported that SPS is under strong genetic control and significantly contributes to final grain number per spike. Genotypes G5 and G8 outperformed others, suggesting their potential as parents in breeding programs aimed at improving spike fertility (Hemshrot et al., 2019, Watt, 2020).

Spike length (SL) varied between 8.8 cm (G7) and 10.5 cm (G8), with a mean of 9.76 cm, SD of 0.53, and a CV of 5.43%. The moderate variability suggests reasonable diversity for selection. According to (Barral Baron, 2016, Desta et al., 2024), spike length is positively correlated with the number of spikelets and grains per spike, as reflected in the higher values observed for genotypes G5 and G8, which also had higher SPS and NGS. Number of grains per spike (NGS) showed substantial variation from 47.6 (G7) to 58.2 (G8), with a mean of 52.82, SD of 3.19, and a CV of 6.04%, the highest among all traits. This high variability implies that NGS is a critical yield component with potential for selection. These results are in agreement with (Amezrou et al., 2018, BELAY and KEBEDE, 2025), who emphasized the role of grain number in yield determination and its sensitivity to both genetic and environmental factors.

The 100-grain weight (HGW) ranged from 3.18 g (G7) to 3.73 g (G8), with a mean of 3.46 g, SD of 0.17, and a CV of 4.91%, indicating moderate variation. Grain weight is a key determinant of final yield and is influenced by assimilate partitioning and grain filling efficiency. These findings are consistent with (Dido et al., 2020, Rahal-Bouziane et al., 2015), who noted that high heritability and moderate variability in 100-grain weight make it a reliable selection index in breeding for improved grain size and density.

Grain yield per plant (GYP) exhibited the broadest range, from 30.6 g (G7) to 36.3 g (G8), averaging 33.26 g, with SD of 1.73 and CV of 5.21%. The observed variability highlights the genetic potential for improving yield in barley through selection. The performance of G8, G5, and G3 was particularly notable, and this trend echoes the findings of [Saeed et al., 2019]. who emphasized the cumulative influence of component traits (PH, SPS, NGS, HGW) on final grain yield (Barral Baron, 2016, Kaur et al., 2019). Overall, the genotypes G8, G5, and G3 consistently outperformed others across several traits, reflecting their genetic superiority and potential as elite lines in barley breeding programs. The observed trait relationships and variability are in line with previous studies emphasizing the value of multivariate and component trait selection in achieving yield gains. The moderate CV values across most traits suggest that these genotypes were evaluated under well-managed field conditions, allowing true genotypic differences to manifest (Tondelli et al., 2013, Zewodu et al., 2025).

Correlation analysis

The correlation analysis among seven agronomic traits in six-row barley genotypes under normal field conditions revealed several significant interrelationships that have important implications for breeding strategies and trait selection (Figure 1). Plant height (PH) exhibited highly significant and positive correlations with flag leaf area (FLA, r=0.92**), spikelets per spike (SPS, r=0.89**), number of grains per spike (NGS, r=0.95**), and grain yield per

plant (GYP, r = 0.89**), indicating that taller plants tended to support greater photosynthetic area and reproductive capacity. These results are in line with the findings of Siahsar et al. (2010), who reported that plant height in barley was positively associated with yield components, particularly in well-watered conditions (Bardehji et al., 2024, Dickin et al., 2012).

Flag leaf area also showed strong positive associations with SPS (r = 0.94**), NGS (r = 0.95**), and GYP (r = 0.88**), suggesting that the flag leaf contributes substantially to assimilate production during grain filling. This supports the conclusions of Ali et al. (2015), who emphasized the role of flag leaf size in determining grain productivity due to its critical function in photosynthesis. Similarly, the strong correlations of SPS with both NGS (r = 0.95**) and GYP (r = 0.91**) indicate that a higher number of spikelets enhances grain set, which in turn increases yield potential. These relationships are consistent with the observations by Kahrizi et al. (2010), who demonstrated that yield improvement in barley can be achieved by selecting for spike-related traits (Kaur et al., 2019, Verma et al., 2021).

Interestingly, spike length (SL) did not show significant correlations with most traits, including GYP (r=0.55~NS), suggesting that SL alone is not a reliable predictor of productivity under normal conditions. This aligns with the findings of (Benlioglu et al., 2025, Bernád et al., 2024), who reported that spike length in barley is often influenced by genetic and environmental variability and may not directly translate to grain number or weight. The number of grains per spike (NGS) exhibited highly significant positive correlations with all major yield-contributing traits, particularly GYP (r=0.94**), reinforcing its importance as a primary determinant of yield. This finding supports earlier studies by (Hemshrot et al., 2019,

Tondelli et al., 2013), who highlighted NGS as a key target for enhancing grain yield in barley breeding programs. On the other hand, 100-grain weight (HGW) showed weaker, non-significant associations with PH (r = 0.43 NS), FLA (r = 0.38 NS), and GYP (r = 0.61 NS), suggesting that while grain size is important, it may not be as strongly influenced by vegetative traits in six-row barley as in other cereal crops. These results are consistent with (Amezrou et al., 2018, Kaur et al., 2019), who reported that grain weight in barley is more genetically independent and may not always contribute significantly to total yield.

Overall, grain yield per plant (GYP) was most strongly and significantly correlated with NGS, SPS, PH, and FLA, indicating that these traits collectively contribute to yield formation through enhanced sink capacity and source strength. The high interdependence of these traits suggests that simultaneous improvement of multiple components—particularly number of grains per spike and spikelet density—can lead to more effective genetic gains. These findings are in agreement with those of (Bensemane et al., 2011, Dickin et al., 2012), who concluded that indirect selection through component traits is a viable strategy for improving grain yield in barley.

The results demonstrate that under normal field conditions, traits like NGS, SPS, FLA, and PH are strongly associated with yield and can serve as effective selection indices in barley breeding programs. While HGW and SL show limited direct impact on yield, they may still be valuable in specific breeding contexts, particularly when targeting grain quality or architecture. These findings emphasize the need for integrative trait selection approaches and support the ongoing development of high-yielding, agronomically superior six-row barley cultivars (Kumar et al., 2020, Watt, 2020).

Table 1. Analysis of variances (ANOVA) for Morphological and Yield Attributes in 10 barley genotypes

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Source of Variation	df	Plant Height (cm)	Flag Leaf Area (cm²)	Spikelets per Spike	Spike Length (cm)	No. of Grains per Spike	100-Grain Weight (g)	Grain Yield per Plant (g)
Replications(R)	2	0.18ns	0.25ns	0.21ns	0.14ns	0.27ns	0.05ns	0.11ns
Genotypes (G)	9	16.62**	5.84**	3.41**	1.57**	30.76**	0.43ns	13.45**
Error	18	0.2	0.23	0.29	0.19	0.33	0.21	0.24
Total	29		_	_	_	_	_	

Table 2. Mean values of Morphological and Yield Attributes of Ten Barley Genotypes Under Normal Growing Conditions

Genotype	Plant Height (cm)	Flag Leaf Area (cm²)	Spikelets per Spike	Spike Length (cm)	No. of Grains per Spike	100-Grain Weight (g)	Grain Yield per Plant (g)
G1	92.1	26.8	20.2	9.6	52.4	3.45	32.7
G2	89.7	25.9	19.6	9.2	49.3	3.32	31.4
G3	95.3	27.5	21	10.1	54.7	3.58	34.6
G4	90.6	26.2	20.1	9.7	51.8	3.39	32
G5	94.4	28	21.3	10.3	56.1	3.62	35.4
G6	91.5	25.7	20.4	9.9	52	3.4	32.9
G7	88.9	24.5	18.8	8.8	47.6	3.18	30.6

G8	96	29.4	22.1	10.5	58.2	3.73	36.3
G9	91.9	26.4	20	9.5	50.7	3.36	31.8
G10	93.7	27.8	21.2	10	55.4	3.59	34.9

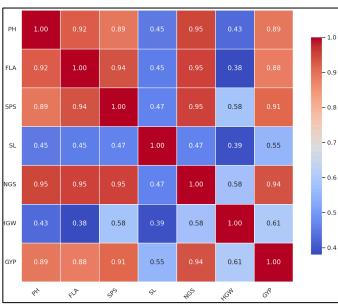


Figure 1. Correlation among Morphological and Yield Attributes of Ten Barley (*Hordeum vulgare* L.) Genotypes Under Normal Growing Conditions

CONCLUSION

This comprehensive evaluation of ten six-row barley genotypes revealed substantial phenotypic variation in key morphological and vield-related traits, offering significant scope for genetic improvement. The observed highly significant differences for plant height, flag leaf area, spikelets per spike, spike length, number of grains per spike, and grain yield per plant confirm the presence of exploitable genetic diversity. Particularly, genotypes G8, G5, and G3 demonstrated superior performance, with G8 exhibiting the highest values for plant height (96.0 cm), flag leaf area (29.4 cm²), spikelets per spike (22.1), number of grains per spike (58.2), and grain yield per plant (36.3 g). The strong positive correlations of grain yield with number of grains per spike (r = 0.94**), spikelets per spike (r = 0.91**), flag leaf area (r = 0.88**), and plant height (r = 0.89**) suggest that these traits contribute synergistically to yield formation. In contrast, 100-grain weight and spike length showed weak or non-significant correlations with yield, indicating limited utility as primary selection indices in this context. The findings support the use of multivariate trait-based selection strategies to enhance breeding efficiency. Overall, the study identifies genotypes with high yield potential and establishes trait relationships that are critical for formulating selection criteria. These insights will aid in the development of high-vielding. agronomically superior barley cultivars suitable for normal production environments.

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