

ARTICLE

# Multi-trait evaluation of barley genotypes under semi-arid conditions using genotype × trait biplot analysis

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**ABSTRACT** Identifying barley genotypes that combine high grain yield with favorable agronomic traits is a key objective of breeding programs. The present study aimed to evaluate a set of barley genotypes and identify superior ones using genotype × trait biplot analysis under rainfed conditions. Twenty barley genotypes were assessed in a field experiment conducted at Moghan, using a randomized block design with three replicates. The biplot analysis explained 68% of the total variation, indicating that the first two principal components effectively captured the complex relationships between genotypes and traits. Genotype G11 emerged as the most superior, demonstrating consistently high performance across a range of yield and yield-related traits, particularly in biomass weight and number of fertile tillers. Genotype G1 (cultivar Mahour) achieved a high grain yield primarily due to an increased thousand-grain weight, reflecting a grain-filling-driven yield strategy. Genotypes G14 and G18 exhibited relatively high yield with low variability, making them stable performers. The polygon view of the biplot distinguished genotypes with specific trait superiority from those showing poor performance, thereby facilitating the efficient elimination of underperforming genotypes such as G3 and G5. The ideal genotype view highlighted G11, followed by G13 and G19, as the genotypes closest to the ideal combination of high mean performance and low variability, confirming their broad desirability across the evaluated traits. Trait association analysis revealed strong positive correlations between biomass weight, number of fertile tillers, and tillers per plant, underscoring the importance of vegetative growth and sink capacity in yield formation under semi-arid conditions. Straw weight, grain yield per plant, and biomass weight exhibited the highest discriminatory power, while spike height and thousand-grain weight provided less useful information for genotype discrimination. The biplot analysis proved to be a highly effective tool for identifying superior barley genotypes and key selection traits, offering valuable insights for breeding programs targeting drought-prone environments.

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## INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops worldwide, with an annual global production of approximately 146 million tons from 46 million hectares; least developed countries account for nearly 1.2 million hectares of the total area under barley cultivation (FAOSTAT 2023). Due to its relatively high tolerance to challenging environmental conditions, including salinity, drought, and low humidity, barley is regarded as a strategic crop for semi-arid regions. The release of new barley varieties with rapid adaptation remains essential, particularly under changing climatic conditions (Kaseva et al. 2023). In many regions, especially Mediterranean envi-

ronments, drought stress frequently occurs during grain filling, coinciding with high temperatures. Late-season drought and heat stress are among the main constraints limiting barley productivity; therefore, identifying high-yielding genotypes with superior morphophysiological traits and enhanced drought tolerance is a major objective of barley breeding programs (Barati et al. 2020). A primary goal in arid and semi-arid regions is to develop varieties that sustain acceptable yields under rainfed conditions, while minimizing yield losses relative to those achieved under optimal irrigation. Achieving such genotypes can improve productivity and water-use efficiency and reduce the waste of limited water resources while maintaining grain yield and quality (Elsayed et al. 2025).

Among abiotic stresses, drought can significantly

reduce yield performance and key agronomic traits, including thousand-grain weight, plant height, spike length, biological yield, grains per spike, number of fertile tillers, and grain weight per spike. Drought stress at various growth stages disrupts physiological processes, especially during grain formation and filling, ultimately resulting in reduced yield (Nyaupane et al. 2024). Although barley is generally more drought tolerant than many other cereal crops, it is highly sensitive to water deficit during critical developmental stages, particularly grain filling, when drought can cause substantial yield losses. Studies evaluating barley genotypes under stress and non-stress conditions have shown that both grain yield and biological yield are significantly reduced under drought (Ferioun et al. 2025; Töpfer et al. 2025). Grain weight reductions under drought have been reported to exceed 50%, while thousand-grain weight reductions range from 21% to 32%, and grain yield reductions are about 50% (Berki et al. 2025). Genotypic differences in response to drought are often significant, whereas the intensity and duration of drought remain unpredictable. Drought is among the most widespread abiotic stresses limiting crop production worldwide.

Plant resistance to drought stress is a complex trait governed by several morphological, physiological, and agronomic characteristics. True drought resistance is reflected in the ability of crops to tolerate water deficit with minimal damage and yield loss (Haghpanah et al. 2024). Accordingly, indirect selection based on drought-related traits has been proposed as an effective strategy for improving grain yield under stress conditions. Several selection criteria and multi-trait indices have been introduced to identify genotypes with both high yield potential and drought tolerance, particularly in advanced breeding generations. Previous studies have shown that drought stress significantly influences yield performance and its components in barley. Drought stress causes significant reductions in relative water content, fertile spikes per unit area, grains per spike, thousand-grain weight, grain yield, and biomass (Hebbache et al. 2024), while increasing ion leakage, proline accumulation, and the activity of antioxidant enzymes such as catalase and guaiacol peroxidase in flag leaves (Mohagheghian et al. 2025). Some traits, including plant height, peduncle length, rachis length, and thousand-grain weight, could serve as useful indicators for selecting high-yielding barley cultivars under both stress and non-stress conditions (Hemadesh et al. 2021).

The genotype  $\times$  trait (GT) biplot has emerged as a powerful multivariate tool for evaluating genotype performance across multiple traits simultaneously. It illustrates relationships among traits, reveals antagonistic or synergistic associations, and clarifies genotype-trait profiles by highlighting genotypes with superior performance

and desirable trait combinations (Hassani et al. 2023). The GT biplot is founded on the principle that yield is a primary trait and strengthens the contribution of other traits by evaluating them in relation to yield, helping to avoid the selection and recommendation of low-yielding genotypes. Moreover, the graphical output facilitates visualization, comparison, and ranking of genotypes based on multi-trait performance (Stansluos et al. 2023). Therefore, the aim of the present study was to apply the GT biplot approach to barley genotypes grown under rain-fed, semi-arid conditions and to identify superior genotypes by integrating all evaluated traits.

## MATERIALS AND METHODS

### *Plant materials*

A total of twenty barley genotypes with diverse agronomic and morphological characteristics were provided by the Seed and Plant Improvement Institute, Iran (Table 1). These genotypes were selected to represent a wide range of adaptation and yield potential under contrasting water availability conditions.

### *Study area*

The experiment was carried out at the Moghan, Ardabil, Iran (39°38' N, 47°54' E; 32 m a.s.l.). Based on the Köppen-Geiger climate classification, the region is categorized as semi-arid, characterized by hot summers, mild winters, and a short cold season. Long-term climatic data indicate a mean yearly temperature of 15 °C, with a mean yearly rainfall of approximately 335 mm. The region's growing season typically experiences a dry period from late spring to early autumn, with the majority of precipitation occurring in winter and early spring. This irregular rainfall pattern makes the region particularly suitable for rainfed agricultural practices. Physicochemical analyses of field soil revealed that the soil texture was clay loam, with a pH of 7.2, an electrical conductivity of 2.4 dS m<sup>-1</sup>, and the soil contained 0.9% organic matter. These characteristics indicate moderate fertility with well-drained conditions suitable for barley cultivation under rainfed conditions. The cropping season was characterized by relatively low rainfall during the growing period, with the majority of precipitation occurring before and after the sowing window. Given the semi-arid nature of the region, the barley genotypes were exposed to water stress, especially during the critical phases of flowering and grain filling.

### *Experimental design*

The experiment was conducted using a randomized complete block design (RCBD) with three replications; each plot represented a genotype evaluated under rainfed

**Table 1.** The characteristics of barley genotypes under rainfed circumstances.

#	Pedigree
G1	Mahour
G2	CBSS97M00850T-G-2M-1Y-2M-0Y
G3	CB06M00193T-C-9M-0AP-0AP-0AP
G4	CBSS07Y00696S-0AP-0AP
G5	CBSS05M00256S-2M-0Y-0M-0AP-0TR
G6	CBSS97M00850T-G-2M-1Y-2M-0TR
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-4AP
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	TR145/195039
G20	M91178002/MAHIGAN

conditions. The field was prepared by deep plowing followed by disking to create a uniform seedbed. Sowing was carried out manually during the second week of November, and all plots were sown on the same date. Each experimental plot measured 1.8 × 3 m and consisted of six rows spaced 30 cm apart. Within each row, plants were spaced 10 cm apart, resulting in approximately 180 plants per plot. To minimize border effects and ensure uniform sampling, data collection was restricted to the central four rows of each plot. Standard agronomic practices for barley production in the region were followed, including fertilization and pest and disease control as required. No supplemental irrigation was provided throughout the growing season.

#### Trait evaluation

At physiological maturity (BBCH 89), when most plants had reached full ripeness, ten randomly selected plants per replicate were harvested to measure agronomic traits. Plant height (PH) was measured from the soil surface to the tip of the main stem, and spike height (SH) was recorded from the soil surface to the top of the spike. The number of tillers per plant (NTP) and the number of fertile tillers (NFT) were counted for each sampled plant, where NFT represents tillers that produced viable seeds. Straw weight (SW) was measured as the dry weight of plant material excluding grain, and the number of grains per plant (NGP)

was counted for each sampled plant. Grain yield per plant (GYP) was determined as the total grain weight of each sampled plant. Biomass weight (BW) was recorded as the total aboveground dry matter from the central rows of each plot harvested at maturity. Grain yield (GY) was determined by harvesting the central rows of each plot at maturity, air-drying the material, and recording the total grain weight.

#### Genotype × trait biplot

The normality of the dataset was evaluated via the Ryan-Joiner procedure. To investigate the pattern of genotype × trait interaction and to provide a graphical interpretation, the biplot methodology was applied. This approach is based on principal component analysis with symmetric scaling of accession and trait scores, and can be expressed as:

$$\frac{x_{ij} - X_j}{S_j} = \sum_{n=1}^2 \lambda_n \alpha_{in} \beta_{jn} + \epsilon_{ij}$$

where  $x_{ij}$  is the value of genotype  $i$  for trait  $j$ ;  $X_j$  is the mean of trait  $j$ ;  $S_j$  is the square root of the variance of trait  $j$ ;  $\lambda_n$  is the eigenvalue associated with the  $n$ -th principal component;  $\alpha_{in}$  and  $\beta_{jn}$  are the scores of genotype  $i$  and trait  $j$  on component  $n$ , respectively; and  $\epsilon_{ij}$  is the residual term not explained by the first two components.

Scaling option 1 was applied to standardize the data by the square root of the variance, accounting for differences in measurement scales among traits. Centering option 2 was used to center the data around trait means. Singular value decomposition option 2 was employed to generate singular scores categorized into trait eigenvectors, producing a trait-focused biplot that facilitates visual interpretation of inter-trait relationships (vector view). Singular value decomposition option 1 was then used to obtain singular scores categorized into genotype eigenvectors, generating a genotype-focused biplot to assess associations and similarities among genotypes (ideal accession view).

## RESULTS

The genotype × trait biplot accounted for 68% of the total variation (Fig. 1), indicating that the first two principal components captured most of the multi-trait pattern. The ANOVA revealed significant genotypic effects for all traits except the number of grains per plant (NGP) and plant height (PH) (Table 2), and coefficients of variation were acceptable. Mean comparisons showed that grain yield (GY) was highest in G1, G2, G4, G11, and G14, whereas

**Table 2.** The analysis of variance for measured traits of barley genotypes.

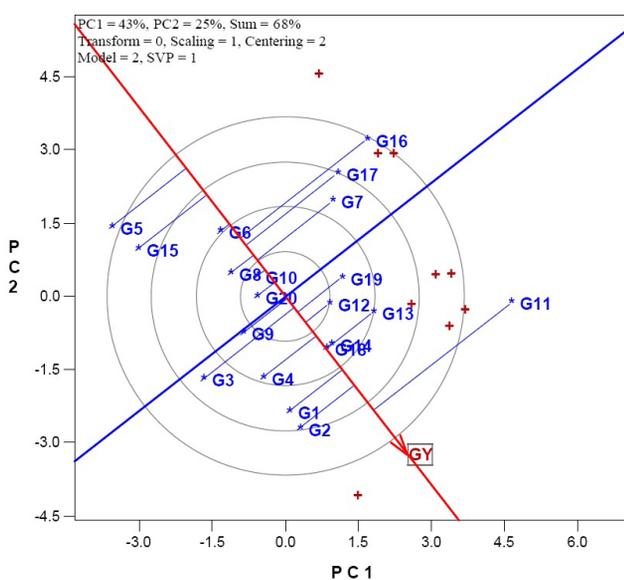
SOV	GY	BW	SW	GYP	NGP	TGW	NTP	NFT	SH	PH
Replication	68526.7 <sup>ns</sup>	529473.9 <sup>ns</sup>	72485.70 <sup>ns</sup>	0.451 <sup>*</sup>	163.80 <sup>ns</sup>	61.39 <sup>**</sup>	0.037 <sup>ns</sup>	0.590 <sup>**</sup>	0.068 <sup>ns</sup>	1162.10 <sup>**</sup>
Genotype	458956.1 <sup>**</sup>	196626123.6 <sup>**</sup>	8898614.20 <sup>**</sup>	0.190	186.15 <sup>ns</sup>	22.63 <sup>**</sup>	0.621 <sup>**</sup>	0.223 <sup>**</sup>	1.062 <sup>**</sup>	113.31 <sup>ns</sup>
Error	39600.4	815368.4	631494.70	0.141	126.61	6.51	0.265	0.109	0.292	120.01
CV§	9.4	11.9	14.6	21.0	20.4	7.9	17.8	14.8	9.2	13.8

Traits are GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height. § CV, coefficient of variation. \*\* and ns are significant at 0.01 statistical level and non-significant.

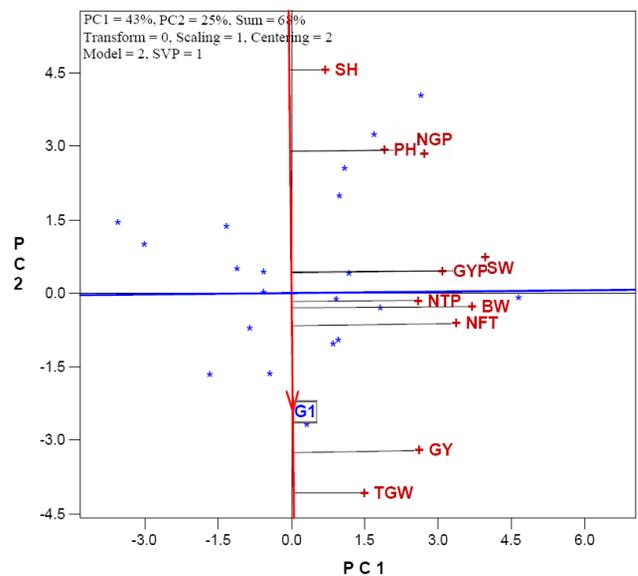
biomass weight (BW) and straw weight (SW) were highest in G11, G13, G14, G16, and G19 (Table 3). Grain yield per plant (GYP) was high for all genotypes except G3, G10, and G15, while no significant differences were detected among genotypes for NGP. Thousand-grain weight (TGW) was high for G1, G2, G3, G4, G9, G11, G12, G13, G14, G18, and G19, and the number of tillers per plant (NTP) was high for G1, G2, G11, G12, and G17 (Table 3). The number of fertile tillers (NFT) was high in G1, G2, G7, G10, G11, G12, G17, G18, and G20, whereas spike height (SH) was highest in G16 and G17; no genotype was clearly superior for plant height (PH) (Table 3). In the biplot, genotypes G11, G1, and G2 (followed by G4, G13, G14, and G18) produced the highest yields (Fig. 1). The proximity of G14 and G18 to the vertical axis suggested relatively stable performance, whereas G11, G16, and G17 showed greater variability (Fig. 1). Overall, G1 and G2, as well as G14 and G18, maintained high yield potential and appear suitable for further evaluation in cultivar

development programs. Notably, G1 corresponds to the cultivar Mahour, indicating that improved lines may be well adapted to semi-arid upland regions of Iran. The interpretation of the GT biplot is consistent with earlier reports in barley, where biplot analysis has been applied to elucidate trait relationships and characterize superior genotypes based on multi-trait performance (Kendal 2020; Karahan and Akgün 2020). Similarly, Sabaghnia et al. (2016) demonstrated that the GT biplot effectively summarizes complex trait interrelationships and facilitates the identification of genotypes with favorable trait combinations.

The performance of G1 (cultivar Mahour) across traits is shown on Fig. 2. Genotype G1 performed above average for thousand-grain weight (TGW) and grain yield (GY), but below average for spike height (SH), plant height (PH), and number of grains per plant (NGP). For the remaining traits (NFT, BW, NTP, GYP, and SW), its performance was close to the overall mean. Therefore,



**Figure 1.** Evaluation of grain yield (GY), across 20 barley genotypes.



**Figure 2.** Examination of genotype G1 across measured traits of barley.

**Table 3.** The means of measured traits and related Tukey's honestly significant difference (HSD) values.

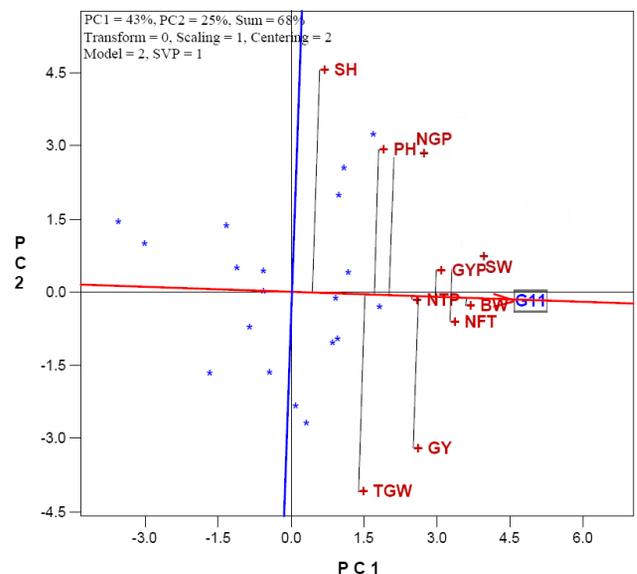
	GY	BW	SW	GYP	NGP	TGW	NTP	NFT	SH	PH
G1	2696.67	6631.71	3935.04	1.75	49.67	35.31	3.13	2.43	5.20	74.93
G2	2700.00	6826.19	4126.19	1.80	52.00	34.61	3.40	2.47	4.87	70.67
G3	2213.33	7160.00	4946.67	1.43	41.00	34.93	2.73	1.83	5.30	79.47
G4	2656.67	7155.33	4498.67	1.83	51.67	35.32	2.80	2.03	6.00	68.93
G5	1386.67	3668.22	2281.55	1.50	50.00	29.99	2.67	1.90	6.40	78.60
G6	2020.00	5128.50	3108.50	1.93	62.67	30.46	2.33	2.13	6.43	78.13
G7	1960.00	5858.00	3898.00	2.22	73.00	30.72	3.07	2.50	6.30	83.53
G8	2160.00	6042.96	3882.96	1.89	64.00	30.02	2.53	2.07	5.93	71.47
G9	2050.00	7400.00	5350.00	1.71	52.00	32.96	2.60	2.13	5.63	72.73
G10	1726.67	8162.00	6435.33	1.30	45.33	28.69	3.07	2.47	5.53	83.33
G11	2696.67	11000.00	8303.33	2.01	58.00	34.58	4.07	2.87	6.20	88.67
G12	1766.67	6656.74	4890.07	1.99	56.33	35.38	3.47	2.60	5.83	80.70
G13	2113.33	9884.06	7770.72	2.14	57.67	36.85	2.53	2.20	5.93	81.90
G14	2423.33	9445.64	7022.31	1.92	56.33	33.81	2.67	2.07	5.27	79.20
G15	1400.00	5687.30	4287.30	1.33	43.67	30.76	2.60	1.77	5.87	86.40
G16	2120.00	9506.31	7386.31	1.99	67.33	28.77	2.80	2.13	7.40	86.60
G17	1893.33	8539.73	6646.40	1.68	61.33	27.94	3.67	2.27	6.73	86.00
G18	2293.33	8349.28	6055.94	1.87	53.67	35.08	2.53	2.40	5.27	83.40
G19	2196.67	10074.18	7877.52	1.80	54.67	32.67	2.47	2.13	5.80	87.80
G20	1893.33	8167.72	6274.39	1.61	53.67	30.28	2.67	2.27	5.67	73.40
HSD	473.30	2147.60	1890.00	0.893	26.76	6.07	1.224	0.787	1.285	26.06

Traits are GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height.

the overall desirability of G1 was primarily driven by its higher TGW (Fig. 2). In contrast, the biplot for G11 (Fig. 3) indicated above-average performance for NFT, BW, NTP, GYP, SW, and GY, with relatively greater variability for GY (distance from the horizontal axis). The trait-specific patterns observed for G1 and G11 agree with earlier studies showing that the GT biplot is effective for identifying trait combinations underlying superior genotype performance; high-yielding barley genotypes are often associated with either strong grain-filling traits (e.g., TGW) or enhanced sink-capacity traits (e.g., fertile tillers, biomass, and grain yield per plant) (Zhang et al. 2021; Abdelghany et al. 2025).

To visualize relationships among traits, vectors extending from the origin to each trait were plotted (Fig. 4). Trait associations were inferred from the cosine of the angles between vectors: 0°, 90°, and 180° indicate positive, null, and negative relationships, respectively. Vector length reflects the relative contribution of each trait to the biplot. Strong positive relationships were detected between PH and NGP, between GYP and SW, and among BW, NFT, and NTP, as indicated by acute angles (Fig. 4). Moderate positive relationships were identified between GY and TGW, between SH with PH and NGP, and between (GYP and SW) and (BW, NFT, and NTP) (Fig. 4). In

contrast, SH and TGW showed little association with the group of traits GYP, SW, BW, NFT, and NTP, and SH and

**Figure 3.** Examination of genotype G11 across measured traits of barley.

**Table 4.** Interrelationships among measured traits of barley genotypes.

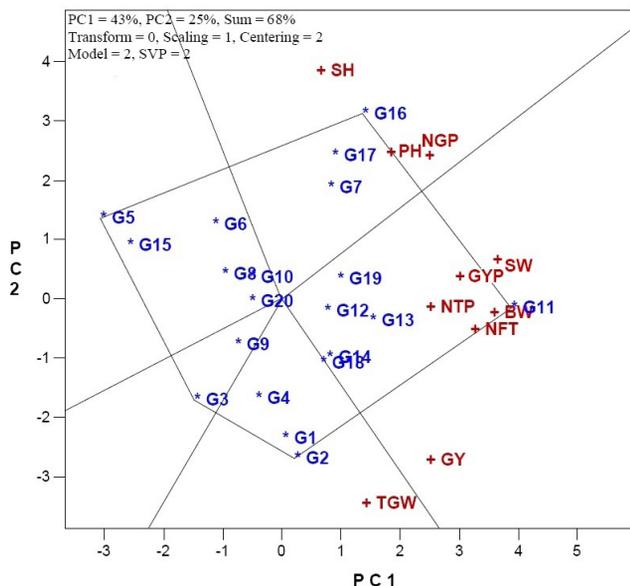
	BW	SW	GYP	NGP	TGW	NTP	NFT	SH	PH
GY	0.44	0.25	0.43	0.08	0.59	0.27	0.37	-0.35	-0.3
BW		0.98	0.28	0.11	0.23	0.25	0.36	0.02	0.44
SW			0.21	0.10	0.12	0.21	0.30	0.10	0.54
GYP				0.8	0.33	0.12	0.44	0.25	0.05
NGP					-0.29	0.10	0.32	0.60	0.16
TGW						0.07	0.21	-0.58	-0.23
NTP							0.73	0.07	0.23
NFT								-0.09	0.18
SH									0.42

Traits are GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height. Critical amounts  $P < 0.05$  and  $P < 0.01$  (with 18 degrees of freedom) are 0.44 and 0.56, respectively.

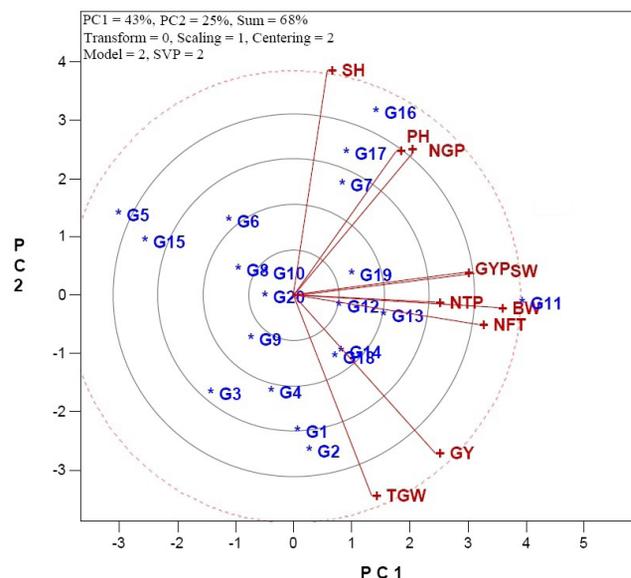
TGW were negatively related to each other as indicated by obtuse angles. Differences between biplot-based and correlation-based results (Table 4) are expected because the biplot summarizes the overall data structure rather than pairwise associations alone.

The trait relationships revealed by the GT biplot in the present study are consistent with earlier genotype  $\times$  trait biplot analyses in barley. Previous studies have shown that acute angles among trait vectors indicate strong positive relationships, particularly among vegetative growth and

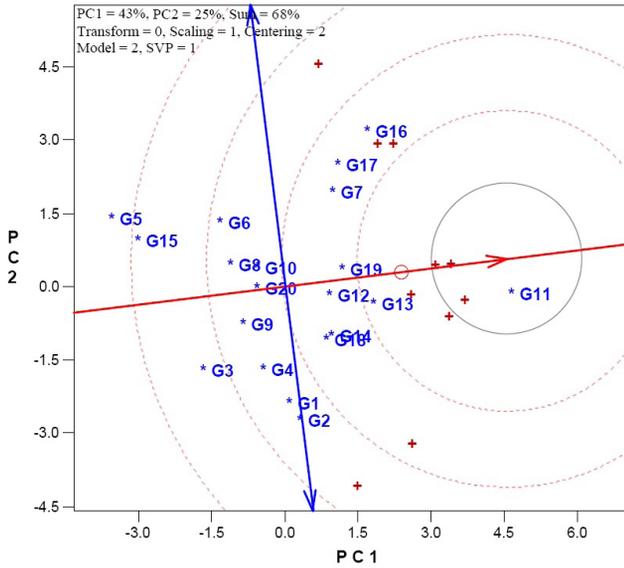
yield-component traits. Bai et al. (2021) and Naeem et al. (2022) reported strong positive associations among biomass weight, fertile tillers, and tillers per plant, like the relationships observed here. Mazurenko et al. (2025) observed comparable grouping of single-plant performance and biological yield, supporting the notion that increased assimilate production enhances per-plant yield; this aligns with the strong positive relationship observed between grain yield per plant (GYP) and straw weight



**Figure 5.** The Polygon of the genotype by trait biplot for identification of traits' relations. Traits are plant height (PH), spike height (SH), number of tillers per plant (NTP), number of fertile tillers (NFT), straw weight (SW), number of grains per plant (NGP), grain yield per plant (GYP), biomass weight (BW), grain yield (GY), and thousand-grain weight (TGW).



**Figure 4.** The vector-view of the genotype by trait biplot for identification of traits' relations. Traits are plant height (PH), spike height (SH), number of tillers per plant (NTP), number of fertile tillers (NFT), straw weight (SW), number of grains per plant (NGP), grain yield per plant (GYP), biomass weight (BW), grain yield (GY), and thousand-grain weight (TGW).

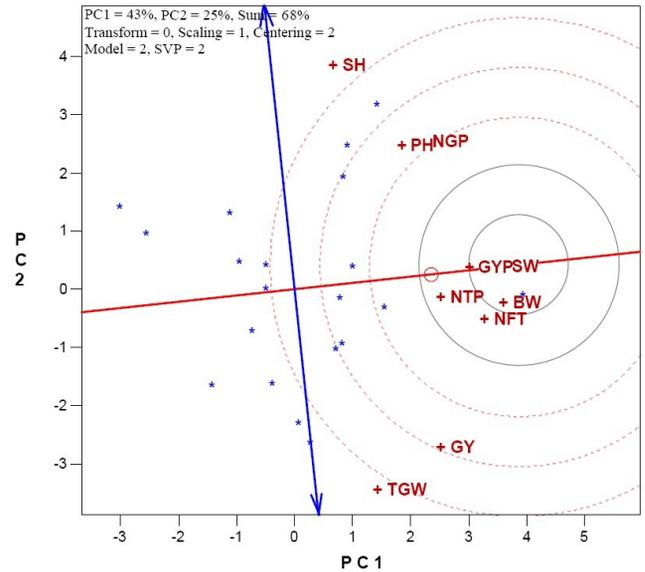


**Figure 6.** The ideal genotype-view of the genotype by trait biplot for identification of traits' relations.

(SW) in our analysis. Previous studies have established positive associations between grain yield and thousand-grain weight in barley (Gasparis and Miłoszewski 2023; Hong et al. 2024), highlighting the role of grain filling in determining final yield. Additionally, Niu et al. (2021) showed that stature traits such as plant height and spike height often form distinct vector orientations relative to yield-component traits. Thus, height-related traits may contribute independently to phenotypic variation rather than directly influencing yield components.

The polygon view of the biplot enabled comparison of genotypes based on trait performance and facilitated the identification of superior genotypes (Fig. 5). A pentagon was formed with genotypes G2, G3, G5, G11, and G16 as vertices. Genotype G2 showed superior TGW; G16 performed best for SH, NGP, and PH; and G11 excelled for the remaining traits (GY, GYP, SW, BW, NFT, and NTP). The remaining vertex genotypes (G3 and G5) did not show superiority for any measured trait, indicating poor overall performance (Fig. 5). Overall, G11 can be identified as the best genotype due to its superior performance for most traits and grain yield. Previous genotype × trait biplot studies have shown that the polygon approach effectively separates genotypes with trait-specific superiority from those with general or poor performance (Sabaghnia et al. 2025). Vertex genotypes often exhibit high performance in one or more key traits, whereas genotypes located within the polygon typically display below-average or moderate trait values (Singhal et al. 2024).

The ideal genotype plot (Fig. 6) identified genotypes



**Figure 7.** The ideal trait-view of the genotype by trait biplot for identification of traits' relations. Traits are plant height (PH), spike height (SH), number of tillers per plant (NTP), number of fertile tillers (NFT), straw weight (SW), grain yield per plant (NGP), grain yield per plant (GY), biomass weight (BW), grain yield (GY), and thousand-grain weight (TGW).

that combined high mean performance across traits with low variability. Genotype G11, followed by G13 and G19, was closest to the ideal genotype, whereas G5 and G15 were the least desirable (Fig. 6). The ideal genotype concept allows breeders to consider both trait magnitude and stability simultaneously, facilitating the identification of broadly superior genotypes. Genotypes located near multiple trait vectors are often considered desirable due to consistent above-average performance across key yield and yield-component traits (Stansluos et al. 2023), a pattern mirrored by G11, G13, and G19 in the present study.

Trait representativeness and discriminating ability are illustrated in Fig. 7 using the ideal trait view. Straw weight (SW), grain yield per plant (GYP), and biomass weight (BW), followed by NFT and NTP, showed the strongest ability to represent and discriminate among genotypes (Fig. 7). In contrast, SH and TGW contributed least in this regard. The identification of informative traits in this study is consistent with previous biplot analyses in barley (Bakhshi and Shahmoradi 2023). Traits with long vectors (strong discriminating ability) and small angles relative to the principal components (high representativeness) are particularly useful for selection. Overall, the GT biplot provided a robust framework for identifying both the most informative traits and the least useful ones, helping to prioritize traits that effectively capture phenotypic variation among genotypes.

## DISCUSSION

The current research utilized the genotype  $\times$  trait biplot approach to evaluate barley genotypes under semi-arid field conditions and to identify superior genotypes based on multi-trait performance. The biplot explained an acceptable proportion of variation (68%), indicating that the first two principal components sufficiently captured the complex interrelationships among genotypes and traits. This level of explained variation is consistent with previous studies utilizing biplot analysis, which typically report 60-75% variance capture as sufficient for reliable interpretation of genotype  $\times$  trait associations (Peixoto et al. 2022; Shirzad et al. 2025). The biplot analysis identified distinct performance patterns among the evaluated barley genotypes. Among the genotypes, G11 consistently emerged as the most superior accession, demonstrating above-average performance for nearly all evaluated traits, including grain yield and biomass weight. The polygon view clearly positioned G11 as a vertex genotype, associated with most yield-related traits, highlighting its strong sink capacity and efficient assimilate partitioning. This finding aligns with earlier reports indicating that high-yielding barley genotypes often derive superiority from combined effects of biomass accumulation and tillering capacity rather than reliance on a single yield component (Roohi et al. 2022; Ahmed et al. 2025).

Genotype G1 (cultivar Mahour) represented a contrasting but equally valuable yield strategy. Whereas G11 excelled across multiple vegetative and reproductive traits, G1 achieved high grain yield primarily through enhanced thousand-grain weight (TGW). The biplot demonstrated the proximity of G1 to the TGW vector, while most other traits were close to average values. These results support the concept that grain yield in barley can be achieved through alternative pathways, either via increased sink number (fertile tillers and grains) or improved grain-filling efficiency (Shirdelmoghanloo et al. 2022; Asad et al. 2023). The balanced performance of G1 across traits further confirms its suitability for semi-arid upland regions, where drought stress during grain filling often favors genotypes with superior kernel weight rather than excessive vegetative growth. Genotypes G14 and G18 also exhibited relatively high and stable grain yield, as indicated by their proximity to the vertical axis of the biplot. Their trait expression showed lower variability across measured characteristics, making them suitable candidates for further multi-location testing. In contrast, genotypes G5, G6, and G15 consistently showed inferior performance, reinforcing the value of GT biplot analysis for early elimination of low-performing germplasm.

The ideal genotype view further refined genotype evaluation by integrating both mean performance and

trait consistency. Genotype G11 was closest to the ideal genotype, followed by G13 and G19. The ideal genotype concept is particularly valuable in breeding programs targeting complex environments, as it identifies genotypes that perform well across multiple traits simultaneously rather than excelling in only one dimension (Vieira et al. 2025). The inferior positioning of G5 and G15 relative to the ideal genotype reinforces their unsuitability for selection due to weak performance across several traits. The biplot vector view revealed important relationships among agronomic traits, providing insights into the mechanisms of yield formation in barley. Strong positive associations were observed between biomass weight, fertile tillers, and tillers per plant, highlighting the central role of tillering and biomass accumulation in determining yield potential. Similar trait groupings have been widely reported in barley and other small-grain cereals, where increased assimilate production supports higher sink capacity and ultimately greater yield performance (Shao et al. 2021; Liang et al. 2023).

The positive relationship between grain yield per plant and straw weight supports the importance of vegetative vigor in sustaining reproductive output; straw weight may reflect stem strength and carbohydrate storage capacity that can be remobilized during grain filling under water-limited conditions. This relationship has been documented in cereals, emphasizing the role of stem reserves in buffering yield losses under water shortage (Dwivedi et al. 2023; Zhang et al. 2024). Positive associations between yield performance and thousand-grain weight highlight the contribution of grain filling to final yield, particularly under late-season drought. Grain-filling efficiency is often less sensitive to moderate water shortage than grain number, making thousand-grain weight a critical trait in rainfed conditions (UnNisa et al. 2022; Ouhemi and Amamou 2024). The biplot distinguished thousand-grain weight as a partially independent yield component, corroborating findings that grain size and grain number may compensate for each other depending on the environment (Serrago et al. 2025). Plant height, spike height, and number of grains per plant formed a distinct group with limited direct association with grain yield, suggesting that stature-related traits contribute more to phenotypic diversity than to yield determination itself. Previous studies have similarly reported weak or non-significant relationships between height traits and yield components, particularly in modern barley germplasm where lodging resistance and harvest index have been optimized (Niu et al. 2021; Liaqat et al. 2024).

The ideal trait view of the biplot identified straw weight, grain yield per plant, and biomass weight as the most informative traits, exhibiting both strong discriminating ability and high representativeness. These traits

effectively captured phenotypic variation among genotypes, making them valuable indirect selection criteria in breeding programs. The importance of biomass-related traits in distinguishing barley genotypes has been emphasized in previous studies, particularly under water-limited conditions where assimilate production influences yield (Mutanda and Figlan 2025; Soleimani et al. 2025). The results have important implications for barley improvement under semi-arid conditions, and the identification of genotype G11 as a broadly superior genotype with high biomass production, strong tillering capacity, and high grain yield suggests its potential as a parental line in breeding programs targeting rainfed circumstances. Its balanced trait profile indicates resilience, key attributes for coping with climatic variability. Genotype G1 (cultivar Mahour) was suitable for environments where late-season drought limits sink development. The coexistence of these contrasting yield strategies within the evaluated germplasm highlights the importance of maintaining genetic diversity in breeding programs to address diverse stress scenarios.

## CONCLUSION

Genotype G11 was identified as the most superior, demonstrating consistently high performance across grain yield and most other traits. Genotype G1 (cultivar Mahour) achieved high grain yield primarily through enhanced thousand-grain weight, representing an alternative yield formation strategy focused on superior grain filling rather than an increased number of sinks. Trait association analysis revealed strong positive relationships among biomass weight, number of fertile tillers, and tillers per plant, underscoring the importance of vegetative growth and sink capacity in barley yield formation under rainfed conditions.

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