

DROUGHT-INDUCED TRAIT CORRELATIONS IN BARLEY GENOTYPES THROUGH A MULTI-TRAIT SELECTION APPROACH

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Abstract. Drought stress significantly influences trait correlations in barley genotypes, affecting overall plant performance and productivity. This study evaluated 30 barley genotypes for various physio-morphological traits under normal and drought conditions using a randomized complete block design (RCBD). Analysis of variance (ANOVA) revealed highly significant ($P < 0.01$) genotypic, environmental, and genotype-by-environment ($G \times E$) interactions for key traits, demonstrating substantial genetic variability and the impact of drought on trait expression. Chlorophyll Content (CC) declined from 18.21 to 17.15 under drought, while Flag Leaf Area (FLA) decreased from 37.81 cm² to 30.51 cm². Plant Height (PH) reduced from 92.07 cm to 82.95 cm, and Biological Yield (BY) declined from 33.03 g to 30.61 g. Main Spike Grain Weight (MSGW) showed a notable reduction from 3.32 g to 2.76 g. Some genotypes demonstrated the ability to perform better and maintain their yield-related traits under water-limited conditions such as G2, G3, G7, G13 and G22 which are critical indicators of drought resilience. Correlation analysis highlighted strong trait associations, with CC positively correlated with BY ($r = 0.69$), NTP with PH ($r = 0.66$), and FLA with MSGW ($r = 0.73$) under normal conditions. Under drought stress, CC showed positive correlations with SL ($r = 0.64$) and MSGW ($r = 0.56$), while NTP was significantly associated with BGW ($r = 0.72$). The stability of traits such as Spike Length (SL) and Number of Tillers per Plant (NTP) across environments suggests their potential as reliable selection criteria in drought-resilience breeding. These findings underscore the importance of understanding drought-induced trait correlations to enhance selection efficiency in barley breeding programs.

Keywords: *yield, morphological, variability, breeding, resilience*

Introduction

Drought stress is a major environmental constraint affecting global crop production, particularly in arid and semi-arid regions. Barley (*Hordeum vulgare* L.),

one of the most widely cultivated cereal crops, is highly valued for its adaptability to diverse environmental conditions (Benlioglu et al., 2024; Subhani et al., 2015). However, its productivity is significantly impacted by water scarcity, necessitating a deeper understanding of drought-induced responses at the physiological and morphological levels. In particular, analyzing trait correlations under drought stress is essential for identifying key characteristics that contribute to yield stability and stress adaptation (Hejazi and Panahi, 2025). A multi-trait selection approach can provide insights into the relationships among different agronomic traits, enabling more efficient breeding strategies for drought resilience (Mohi-Ud-Din et al., 2024; Khalili et al., 2016).

Drought stress affects various physiological and morphological traits in barley, leading to reduced chlorophyll content, diminished leaf area, stunted plant height, and lower biomass accumulation. These changes directly impact yield-related traits such as spike fertility, grain weight, and overall productivity. Understanding how these traits interact under water-limited conditions is critical for developing stress-tolerant genotypes (Slawin et al., 2024; Baloch et al., 2024). Correlation analysis plays a pivotal role in identifying stable and drought-resilient traits by revealing the interdependence of different physiological and morphological characteristics. For instance, chlorophyll content is often associated with biomass production, while traits like spike length and tiller number contribute to grain yield stability under stress conditions (Findurová et al., 2023; Ghavidel et al., 2024).

The correlation among yield and yield-related traits in barley under drought conditions is critical for developing drought-tolerant varieties. Research indicates significant associations between grain yield and various yield related-traits under both irrigated and stressed conditions. Key yield-related traits in barley that correlate with drought tolerance include chlorophyll content, grain yield, biological yield, harvest index, kernel weight, and days to heading (Mansour et al., 2017; Kalaji and Guo, 2008). Additionally, dominant gene action plays a significant role in influencing yield-related traits, complicating early selection efforts. The identification of specific genotypes exhibiting high drought tolerance and yield stability further emphasizes the potential for breeding programs to enhance barley's resilience to water stress (Mansour et al., 2017; Slawin et al., 2024; Khan et al.).

Conventional breeding approaches for drought tolerance typically focus on single-trait selection, which may not capture the complex interactions between multiple agronomic traits. A multi-trait selection approach, however, considers the genetic associations among traits, improving selection efficiency and accelerating the development of drought-resilient barley cultivars (Bresta et al., 2018; Fatemi et al., 2023). By integrating correlation analysis into breeding programs, researchers can prioritize traits that exhibit strong associations with yield stability under drought stress, thereby enhancing selection accuracy (Hasanuzzaman et al., 2019; Afzal et al., 2024).

By leveraging a multi-trait selection approach, this study contributes to the broader objective of improving barley breeding strategies for enhanced drought resilience. Understanding these trait correlations is essential for developing high-yielding barley cultivars capable of sustaining productivity in water-limited environments, ensuring food security in drought-prone regions (Findurová et al., 2023; Ijaz et al., 2023; Kadege et al., 2024). Drought resilience and yield are quantitative traits influenced by multiple genes and their interactions. Therefore, a multi-trait selection approach was

used in this study to assess the relationships among key physiological and morphological traits, ensuring an effective strategy for identifying drought-tolerant barley genotypes (Hasanuzzaman et al., 2019; Jouyban et al., 2015; Mohi-Ud-Din et al., 2024). This study aims to evaluate the correlation patterns of key physio-morphological traits in barley genotypes under normal and drought conditions. In this study, 30 barley genotypes were assessed for their performance under contrasting moisture conditions. The findings of this research will provide valuable insights into how drought-induced changes in one trait influence the expression of others, ultimately aiding in the identification of optimal trait combinations for stress tolerance.

Materials and methods

The experiment was conducted in November 2023 at the experimental area of the Department of Plant Breeding and Genetics (PBG), The Islamia University of Bahawalpur, Punjab, Pakistan (29.24°N, 71.41°E). A total of 30 barley genotypes were grown to evaluate various physio-morphological traits under drought and normal conditions using randomized complete block design with three replications (*Table 1*). Five plants per genotype were selected within each replication, and data were averaged to ensure reliable trait evaluation and minimize individual plant variability. These genotypes selected for study due to their genetic diversity, widely cultivated, agronomic performance, and relevance to drought tolerance studies. Ten seeds of each genotype were sown in rows, maintaining a plant-to-plant distance of 6 inches and a row-to-row distance of 12 inches. After germination, thinning was carried out, and five plants from each genotype were selected for further evaluation. All genotypes were sown under both normal and drought conditions. In the normal experiment, irrigation was applied according to recommendations, particularly at critical stages: tillering (35 days after sowing - DAS), booting (85 DAS), and milking (112 DAS). Drought conditions were imposed at the tillering stage by skipping irrigation (Noorka and Teixeira da Silva, 2014). Therefore, genotypes under normal conditions received a total of three irrigations at the three critical stages, while genotypes under drought conditions received only two irrigations (at the booting and milking stages). Standard agronomic and cultural practices were followed throughout the growing season, as per recommendations.

Table 1. List of 30 barely genotypes used in this study

Code	Genotype name	Code	Genotype name	Code	Genotype name
G1	DL-36	G11	Sheikh-638	G21	Neelum
G2	Jau-21	G12	Aaaj-2013	G22	TJ-70
G3	Peral-21	G13	Sultan-17	G23	Barley Dwarf
G4	IC-062189	G14	Jau-E-Paghambri	G24	Clipper
G5	EC-663751	G15	Haider-93	G25	Frontier-87
G6	Haider-93	G16	JAU-83	G26	Frontier-87
G7	Jau-17	G17	Talbina-21	G27	PB-2999
G8	Bajwar-2000	G18	Sanober-96	G28	IB-22
G9	Sheikh-634	G19	Rakhshan-10	G29	BW-57
G10	JAU-87	G20	Sadabhar	G30	IB22-1

Measurement of traits

Chlorophyll content was measured using a Hansatech SPAD meter (Model CL-01), with three readings taken from different points on a single leaf, and the average value was recorded. The number of tillers per plant was recorded at crop maturity from selected plants across replications, and the mean data was used for analysis. Flag leaf area was determined by measuring the minimum and maximum leaf width and length of matured plants, and the area was calculated using the formula (Müller, 1991): Flag Leaf Area = Flag Leaf Length × Flag Leaf Width × 0.74. Plant height was measured from the base to the tip of the selected plants across three replications, and the mean value was used for statistical analysis. The number of spikelets per spike was counted from each selected spike and the mean values were determined for analysis. Peduncle length was measured from the last node to the base of the spike at the maturity stage, and the mean value was used for statistical analysis. Spike length was recorded from the base to the tip of the spike, excluding awns, and the mean spike length was noted. Biological yield, which represents the total plant weight, was measured using an electric balance (Compax-CX-600), and data from selected plants were recorded. The main spike weight was also recorded using an electric balance, and the mean data from selected spikes were used for further analysis. Main Spike Grain Weight was determined by threshing the mother spike from each selected plant, weighing it using an electric balance, and calculating the average grain yield across replications.

Statistical analysis

The analysis of variance (ANOVA) for all studied traits was conducted (Steel and Torrie, 1981). Additionally, Pearson's correlation coefficients were calculated using Statistix 8.1 to assess the relationships among studied traits. The significant traits were further analyzed to determine their interlinkages using Pearson's correlation matrix.

Results and discussion

Variability in traits and genotypes under both normal and drought conditions is essential for developing drought-tolerant wheat cultivars. Studies have demonstrated significant genetic diversity among barley genotypes, with the examined traits exhibiting substantial variation under both conditions, as previously reported (Istanbuli et al., 2020; Jouyban et al., 2015). In the present study, drought stress was imposed by skipping one irrigation at the tillering stage, a critical phenophase that significantly influences plant establishment, tiller production, and overall biomass accumulation. The decision to apply stress at this stage was based on its known impact on yield-related traits, as tillering determines the number of productive shoots, which directly affects grain yield under water-limited conditions (Victoria et al., 2023; Teoh et al.). Additionally, previous studies have demonstrated that drought stress at early growth stages can lead to long-term physiological and morphological adjustments that enhance drought resilience. While it is acknowledged that plants may respond differently to drought stress at various phenophases, such as flowering or grain-filling stages, our focus on the tillering stage allows for the identification of genotypes capable of maintaining productivity under early-season moisture deficits (Hejazi and Panahi, 2025; Ahsan et al., 2024). Analysis of variance revealed that genotype

variances were consistently higher than environmental variances, indicating a strong genetic influence on trait expression. The following section provides detailed insights into our study.

Variability in traits and genotypes under normal and drought conditions

The ANOVA results indicate significant variations among different sources of variation (Environment, Genotype, and Genotype × Environment interaction) for most of the quantitative traits studied (Table 2). The environment had a highly significant ($P < 0.01$) effect on all traits, demonstrating that normal and drought conditions significantly influenced barley genotypic performance. This highlights the impact of environmental stress on trait expression. Genotypic differences were highly significant ($P < 0.01$) for all traits, indicating substantial genetic variability among the 30 barley genotypes. This suggests the presence of exploitable genetic diversity for trait improvement (Desta et al., 2024; Hejazi and Panahi, 2025). The $G \times E$ interaction was significant ($P < 0.01$) for most traits, such as Chlorophyll Content (CC), Flag Leaf Area (FLA), Plant Height (PH), Peduncle Length (PL), Biological Yield (BY), Main Spike Weight (MSW), and Main Spike Grain Weight (MSGW). This suggests that genotypic performance varied across environmental conditions, emphasizing the need for stability analysis in genotype selection. However, traits like Number of Tillers per Plant (NTP), Spikelets per Spike (SPS), and Spike Length (SL) showed non-significant interactions, indicating their relative stability across environments. The relatively small error mean squares indicate reliable data quality and precision in trait evaluation. The findings of current study suggest that both genetic and environmental factors significantly influence trait expression in barley. The significant $G \times E$ interactions highlight the necessity for multi-environment trials to identify stable and high-yielding genotypes suitable for drought-prone conditions (Moualeu-Ngangué et al., 2020; Kalaji and Guo, 2008; Mansour et al., 2017).

Table 2. Mean sum of square values for quantitative traits of 30 barley genotypes under normal and drought conditions

Source	Replication	Environment	Genotype	ENV*Gen	Error	Total
DF	2	1	29	29	28	89
CC	249.21	162.65**	46.13**	9.86**	5.922	
NTP	13.749	26.7548**	7922**	3.1699 ^{ns}	3.2556	
FLA	81.69	7768.87**	171.36**	39.49**	25.93	
PH	219.1	14806.1**	421.8**	234.4*	190	
SPS	509.81	533.05**	20.28**	4.86 ^{ns}	4.43	
PL	3.79	143.46**	28.09**	7.1**	4.46	
SL	1173.06	106.17**	9.36**	5.41 ^{ns}	4.76	
BY	1.189	748.01**	538.179**	132.098**	1.037	
MSW	0.656	336.676**	3.215**	0.846**	0.056	
MSGW	0.05	323.70**	3.17**	0.92**	0.02	

DF = Degree of freedom, SS = Sum of squares, MS = Mean Squares, * = $P < 0.05\%$, ** = $P < 0.01\%$, CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelets per Spike), PL (Peduncle Length), SL (Spike Length), BGW (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight)

Traits like Number of Tillers per Plant (NTP) and Spike Length (SL) showed non-significant interactions, indicating their relative stability and potential as selection criteria for broad adaptability. The presence of significant genetic effects ($P < 0.01$) across all traits underscores the role of genetic control in trait expression, which can be exploited through breeding strategies. In our study, Number of Tillers per Plant (NTP) and Spike Length (SL) exhibited non-significant genotype-by-environment ($G \times E$) interactions, indicating their stability across contrasting moisture conditions. This suggests that these traits may be controlled by major genes with minimal environmental influence, aligning with previous research on traits exhibiting high heritability and genetic control under stress conditions (Istanbuli et al., 2020; Khan et al., 2021; Mohi-Ud-Din et al., 2024). While it is true that traits governed by major genes might have limited direct influence on adaptability, their stability across diverse environments makes them valuable as selection criteria in breeding programs. Specifically, stable traits can serve as indicators of genetic resilience and contribute indirectly to adaptability by maintaining yield-related attributes under drought stress (Saidi et al., 2024; Slawin et al., 2024). Furthermore, the relatively low error mean squares enhance the reliability of these findings, suggesting precise data collection. These results support the necessity of multi-environment trials and marker-assisted selection to develop barley cultivars with improved yield stability and stress tolerance under changing climatic conditions (Khalili et al., 2016; Mansour et al., 2017).

The descriptive statistical analysis of 30 barley genotypes under both normal and drought conditions provides critical insights into the impact of water stress on key agronomic and yield-related traits (*Table 3*). A comparison between the two environments reveals significant reductions in most traits under drought conditions, indicating the strong influence of water availability on barley growth and productivity (Hasanuzzaman et al., 2019; Hejazi and Panahi, 2025). Chlorophyll Content (CC), a key indicator of photosynthetic efficiency and stress tolerance, ranged from 10.93 to 25.94 under normal conditions, with a mean of 18.21, whereas under drought, it ranged from 10.77 to 24.34, with a lower mean of 17.15. The coefficient of variation (CV) was slightly reduced under drought (18.80% \rightarrow 15.57%). The results were in line with some previous research suggesting that while chlorophyll content declined, some genotypes were able to maintain their chlorophyll stability, which is crucial for sustaining photosynthesis under stress (Kalaji and Guo, 2008; Irshad et al., 2024).

Number of Tillers per Plant (NTP) exhibited a high degree of variability under both conditions, with means of 6.44 in normal conditions and 6.05 in drought. The CV remained high (26.69% in normal vs. 25.96% in drought), highlighting the sensitivity of this trait to environmental stress and the presence of genotypic variation. Despite a reduction in tiller production, some genotypes showed resilience, making this trait a key selection criterion for drought tolerance. Flag Leaf Area (FLA), an important factor in determining photosynthetic efficiency, decreased significantly under drought, with its mean reducing from 37.81 cm² to 30.51 cm². The CV increased from 15.49% under normal conditions to 19.94% under drought, indicating a greater variability in genotypic responses under water stress. This suggests that leaf area retention under drought could be a valuable selection criterion for drought-tolerant genotypes (Mansour et al., 2017; Moualeu-Ngangué et al., 2020).

Plant Height (PH) exhibited a notable reduction from 92.07 cm under normal conditions to 82.95 cm under drought, reflecting the negative impact of water stress on plant growth. The CV increased slightly (6.16% to 11.03%), suggesting that while some

genotypes maintained their height better, others were more severely affected by drought. Previously (Derbew et al., 2013) found significant variability in plant height and time to maturity among barley genotypes which similar to current findings. Spikelets per Spike (SPS) also showed a reduction in mean values from 20.14 to 18.43 under drought, with a slight increase in variability (CV = 9.47% to 13.07%), indicating that while drought negatively affected spikelet development, some genotypes were better able to maintain their reproductive structures. Peduncle Length (PL) and Spike Length (SL) exhibited reductions in mean values under drought conditions (PL: 13.77 cm → 12.76 cm; SL: 11.93 cm → 9.10 cm). The variability in PL increased slightly (CV = 17.54% to 19.06%), while SL exhibited the lowest variation under drought (CV = 7.57%), suggesting that SL is a relatively stable trait across environments. The barley scientists (Desta et al., 2024) measured the spike length of barley landraces in the range of 6–14.4 cm with an average of 8.73 cm. Similarly, (Hejazi and Panahi, 2025; Istanbuli et al., 2020) reported spike lengths in barley germplasm in the range of 6.5–11.1 cm. The findings of current study which is in agreement with previous research.

Table 3. Descriptive statistics of quantitative traits of 30 barley genotypes under normal and drought conditions

Parameters	Environments	Minimum	Maximum	Mean	Variances	SD	CV
CC	Normal	10.93	25.94	18.21	11.72	3.42	18.80
	Drought	10.77	24.34	17.15	7.13	2.67	15.57
NTP	Normal	2.56	10.33	6.44	2.96	1.72	26.69
	Drought	3.11	9.89	6.05	2.47	1.57	25.96
FLA	Normal	23.36	48.24	37.81	34.30	5.86	15.49
	Drought	18.90	43.23	30.51	37.03	6.09	19.94
PH	Normal	81.27	102.60	92.07	32.15	5.67	6.16
	Drought	60.11	98.36	82.95	83.70	9.15	11.03
SPS	Normal	11.22	23.22	20.14	3.64	1.91	9.47
	Drought	11.81	22.87	18.43	5.80	2.41	13.07
PL	Normal	8.72	19.81	13.77	5.83	2.42	17.54
	Drought	7.33	18.78	12.76	5.92	2.43	19.06
SL	Normal	6.19	17.23	11.93	3.51	1.87	11.07
	Drought	5.73	14.57	9.10	1.49	1.22	7.57
BGW	Normal	20.25	59.28	33.03	107.04	10.35	31.32
	Drought	14.91	56.96	30.61	119.11	10.91	35.65
MSW	Normal	2.80	4.42	3.34	0.64	0.80	9.61
	Drought	1.69	3.19	2.83	0.73	0.86	12.55
MSGW	Normal	1.97	2.88	3.32	0.48	0.70	20.93
	Drought	1.45	2.05	2.76	0.26	0.51	18.41

CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelets per Spike), PL (Peduncle Length), SL (Spike Length), BY (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight), SD (Standard Deviation), CV (Coefficient of variations)

Biological Weight per Plant (BGW), a critical yield-related trait, showed a substantial decline from 33.03 g under normal conditions to 30.61 g under drought. It

exhibited the highest variability among all traits, with its CV increasing from 31.32% to 35.65%, indicating that genotypic differences in biomass production were more pronounced under drought conditions. This highlights BGW as a key trait for selection in drought-tolerant breeding programs. Main Spike Weight (MSW) and Main Spike Grain Weight (MSGW), which directly influence final grain yield, also showed significant reductions under drought. MSGW declined from 3.34 g to 2.83 g, with its CV increasing from 9.61% to 12.55%, while MSW decreased from 3.32 g to 2.76 g, with an increase in variability (CV = 20.93% to 18.41%). The reductions in these traits highlight the negative impact of drought on grain production, though the presence of genotypic variation suggests potential for selecting resilient genotypes. In previous research, diverse ranges of grain weight values in barley genotypes have been reported, such as 17.77–67.23 g, 30.5–58.8 g, and 23.2–52.0 g. In our study, the TGW values of barley germplasm were according to the previous findings (Kalaji and Guo, 2008; Khalili et al., 2016).

The ANOVA results indicate significant variations among environmental conditions, genotypes, and their interactions, highlighting the influence of both genetic and environmental factors on barley trait expression. The environment had a highly significant effect ($P < 0.01$) on all traits, demonstrating the impact of drought on barley genotypic performance. Significant genotype-by-environment ($G \times E$) interactions were observed for key traits such as Chlorophyll Content (CC), Flag Leaf Area (FLA), Plant Height (PH), and Biological Yield (BY), emphasizing the need for multi-environment trials to identify stable genotypes. Under normal conditions, CC ranged from 10.93 to 25.94 (mean = 18.21), while drought reduced it to 10.77–24.34 (mean = 17.15), affecting photosynthetic efficiency. Similarly, PH decreased from 92.07 cm in normal conditions to 82.95 cm under drought, reflecting stress impact. Traits like Number of Tillers per Plant (NTP) and Spike Length (SL) showed non-significant $G \times E$ interactions, with SL exhibiting the lowest variation under drought (CV = 7.57%), suggesting their stability and potential for broad adaptability. The relatively low error mean squares confirm the precision of trait evaluation, supporting the necessity of breeding for drought-tolerant barley cultivars (Khalili et al., 2016; Moualeu-Ngangué et al., 2020; Subhani et al., 2015).

Current findings suggests that certain genotypes respond differently to drought, creating an opportunity for breeding programs to select for drought resilience. Traits such as NTP, BGW, and MSGW, which exhibited high variation, could serve as effective selection criteria for drought-tolerant breeding. Meanwhile, traits like SL and PH, which showed relatively lower variation under drought, indicate stability and may contribute to maintaining productivity under stress (Kalaji and Guo, 2008). These findings emphasize the importance of selecting genotypes with superior biomass production, spike fertility, and grain weight retention to develop high-yielding, drought-resilient barley cultivars. Future breeding strategies should focus on enhancing genetic stability and adaptability to improve barley performance under water-limited conditions (Istanbuli et al., 2020; Jouyban et al., 2015).

Performance of barley genotypes under normal and drought conditions

To assess the genetic variability in barley, the performance of 30 genotypes was evaluated under normal and drought conditions across ten morpho-physiological traits. The analysis highlighted significant differences in trait values, revealing both high-performing genotypes suitable for breeding and those that exhibited poor performance

under stress conditions. *Table 4* provided the mean values of various traits for 30 barley genotypes under normal conditions, assessing them across ten different traits. The top five genotypes exhibiting the highest mean values across these traits included G2, which stood out for its high performance in CC (25.69), FLA (46.59), PH (102.21), and SPS (23.00). G3 followed closely with strong values, particularly in FLA (46.11) and PH (102.20). G7 also demonstrated superior performance, especially in FLA (48.24), PH (102.60), and MSGW (4.50). G13 showed competitive results in FLA (48.24), PH (101.32), and SPS (23.11), while G22 performed consistently well across traits, particularly in FLA (48.24), PH (101.84), and MSGW (4.71). On the other hand, the bottom five genotypes with the lowest mean values were G19, which underperformed in several traits, including CC (11.70), NTP (3.56), and FLA (23.60); G24, which exhibited similarly low values, especially in CC (12.62), NTP (3.33), and FLA (23.39); G28, which ranked low with CC (12.77), NTP (3.44), and FLA (23.70); G4, which showed low results in CC (10.93), NTP (2.89), and FLA (23.36); and G10, which performed poorly with CC (13.17), NTP (3.78), and FLA (23.84). These findings highlighted the significant variability across the barley genotypes, with some exhibiting notably higher values in key traits than others (Findurová et al., 2023; Istanbuli et al., 2020; Khan et al., 2021).

Under drought conditions, *Table 5* showed the mean values of several traits for 30 barley genotypes. The top five genotypes with the highest mean values across the traits were G3, which performed strongly in several traits, including CC (24.34), NTP (8.11), FLA (43.16), PH (98.14), and MSGW (3.86); G2, which demonstrated strong performance with CC (22.80), FLA (43.23), PH (98.14), and MSGW (3.80); G13, which ranked highly with CC (22.35), FLA (43.11), PH (98.22), and MSGW (4.33); G7, which excelled in CC (22.19), FLA (43.16), PH (98.00), and MSGW (3.93); and G22, which rounded out the top five with CC (22.27), FLA (43.23), PH (98.29), and MSGW (4.39). On the other hand, the five genotypes with the lowest mean values included G4, which showed the lowest values in CC (12.55), FLA (19.19), PH (65.44), SPS (12.25), and MSGW (1.96); G19, which performed poorly with CC (10.77), NTP (3.44), FLA (19.23), PH (63.44), and MSGW (1.79); G10, which showed low values in CC (12.55), NTP (3.22), FLA (18.90), PH (63.11), and MSGW (1.83); G24, which exhibited low performance with CC (12.06), NTP (3.11), FLA (19.18), PH (65.33), and MSGW (1.82); and G28, which demonstrated poor performance across traits with CC (13.16), NTP (3.67), FLA (19.09), PH (64.78), and MSGW (1.84). These results highlighted significant variation in the response of barley genotypes to drought conditions. Those genotypes showed higher tolerance and better adaptation to water stress, reflected in their relatively high mean trait values, while others exhibited poor performance across multiple traits. This information was crucial for identifying drought-tolerant genotypes, which could be prioritized in breeding programs aimed at improving drought resistance in barley. The contrasting performance of the genotypes provided valuable insights for selecting the most resilient lines to withstand challenging environmental conditions (Hasanuzzaman et al., 2019; Müller, 1991; Varshney et al., 2012).

In the current study, while drought stress significantly reduced the overall productivity of most barley genotypes, some genotypes demonstrated the ability to maintain their yield-related traits under water-limited conditions such as G2, G3, G7, G13 and G22. Notably, these genotypes exhibited relatively stable values for traits such as Flag Leaf Area (FLA), Biological Yield (BY), Main Spike Grain Weight (MSGW), and Chlorophyll Content (CC), which are critical indicators of drought resilience. These

genotypes maintained higher biomass production, efficient resource allocation, and stable grain weight, suggesting their potential for breeding programs focused on drought tolerance (Benlioglu et al., 2024; Khan et al., 2021; Moualeu-Ngangué et al., 2020).

Table 4. Mean values of studied traits for 30 barley genotypes under normal conditions

Code	CC	NTP	FLA	PH	SPS	PL	SL	BGW	MSW	MSGW
G1	16.87	6.89	32.81	87.70	20.33	12.14	16.87	35.84	6.11	3.69
G2	25.69	9.89	46.59	102.21	23.00	19.04	29.23	59.28	9.41	4.38
G3	25.94	10.00	46.11	102.20	23.22	19.81	20.11	57.72	9.42	4.37
G4	10.93	2.89	23.36	82.27	16.33	8.72	14.91	20.56	5.80	2.34
G5	13.88	6.33	31.42	91.10	20.56	13.98	16.74	25.46	8.41	3.53
G6	16.59	6.89	39.17	86.90	19.89	13.67	15.41	29.33	8.10	3.61
G7	25.56	10.33	48.24	102.60	23.22	19.44	20.22	58.81	9.23	4.5
G8	16.27	8.11	36.73	91.06	19.44	13.68	15.63	20.77	8.42	2.76
G9	16.84	6.22	30.63	87.33	20.67	14.50	15.03	31.28	8.18	3.48
G10	13.17	3.78	23.84	81.27	16.78	9.32	14.19	20.77	6.40	2.54
G11	25.13	6.33	38.40	93.67	21.22	15.56	16.78	53.11	8.43	2.69
G12	21.76	5.78	43.36	92.65	21.89	11.00	15.72	29.41	8.31	2.75
G13	25.26	10.00	48.24	101.32	23.11	19.41	20.00	57.90	9.30	4.28
G14	18.44	7.89	40.08	91.45	20.33	15.91	16.60	22.64	8.65	3.38
G15	17.19	6.44	44.38	95.88	21.44	11.91	18.74	39.20	9.42	4.1
G16	16.00	5.11	41.87	87.75	18.78	13.73	16.01	27.03	8.29	3.57
G17	13.81	4.67	39.90	88.40	19.22	13.60	16.28	30.09	8.31	2.34
G18	15.51	5.11	43.65	90.87	19.00	15.17	17.94	30.63	8.87	3.39
G19	11.70	3.56	23.60	82.41	16.78	10.09	14.68	20.25	6.11	2.66
G20	13.34	5.11	41.41	87.89	19.67	11.83	16.09	28.53	8.31	3.16
G21	16.52	5.67	42.64	88.46	19.56	13.94	15.77	31.02	8.36	2.61
G22	25.51	9.89	48.24	101.84	23.00	19.44	21.54	58.36	9.29	4.71
G23	16.94	5.67	40.17	90.39	19.22	15.22	17.44	31.09	9.17	2.77
G24	12.62	3.33	23.39	82.38	16.78	9.36	14.99	20.50	6.04	2.61
G25	15.70	5.11	40.59	95.82	17.44	18.06	17.51	39.03	8.55	2.71
G26	14.07	4.78	38.39	94.80	19.44	14.47	16.36	39.59	9.14	4.3
G27	17.49	7.56	34.73	99.81	20.11	15.80	17.21	23.52	8.57	2.78
G28	12.77	3.44	23.70	82.89	16.33	10.07	14.67	20.53	6.47	2.51
G29	14.07	7.78	37.75	99.41	21.00	14.83	19.63	34.48	9.11	3.05
G30	14.68	6.33	38.82	100.12	20.78	17.11	17.00	32.60	9.12	2.91

CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelets per Spike), PL (Peduncle Length), SL (Spike Length), BGW (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight)

Correlation under normal conditions

The Pearson's correlation analysis among different quantitative traits of barley genotypes under normal conditions provides insights into the interrelationships between agronomic and yield-related traits, which are crucial for effective selection in breeding programs (Fig. 1). Chlorophyll Content (CC) exhibited significant positive correlations

with most traits, including Number of Tillers per Plant (NTP) ($r = 0.67$), Biological Yield (BY) ($r = 0.69$), and Flag Leaf Area (FLA) ($r = 0.53$), suggesting that higher chlorophyll content contributes to improved tillering and biomass production. Its positive correlation with Main Spike Grain Weight (MSGW) ($r = 0.43$) indicates that photosynthetic efficiency plays a vital role in grain formation (Kalaji and Guo, 2008; Slawin et al., 2024).

Table 5. Mean values of studied traits for 30 barley genotypes under drought conditions

Code	CC	NTP	FLA	PH	SPS	PL	SL	BGW	MSW	MSGW
G1	14.85	8.56	29.83	80.89	19.53	11.28	14.36	47.84	6.73	2.78
G2	22.80	8.56	43.23	98.14	20.92	17.50	18.28	54.30	8.19	3.80
G3	24.34	8.11	43.16	98.14	20.97	17.50	18.34	56.96	7.92	3.86
G4	12.55	3.33	19.19	65.44	12.25	7.33	13.73	15.52	5.21	1.96
G5	14.33	7.56	28.85	85.67	19.86	12.50	15.38	38.78	6.58	2.91
G6	15.52	6.78	30.90	76.78	19.58	14.83	13.73	30.89	7.02	1.88
G7	22.19	8.22	43.16	98.00	22.08	18.78	18.57	55.67	7.98	3.93
G8	14.85	5.56	32.07	91.22	19.58	11.11	16.44	15.03	7.36	1.98
G9	18.14	5.22	32.38	86.11	18.64	14.80	15.53	31.11	6.82	2.61
G10	12.55	3.22	18.90	63.11	12.36	9.72	14.18	15.03	5.00	1.83
G11	16.67	6.78	27.14	85.00	19.25	13.89	17.49	23.68	7.29	2.85
G12	18.01	8.22	26.36	83.11	20.25	14.97	17.54	39.89	7.40	2.65
G13	22.35	9.89	43.11	98.22	20.81	17.48	18.44	54.88	7.91	4.33
G14	14.49	5.78	24.59	82.56	20.14	9.78	16.84	15.21	7.41	3.22
G15	19.08	6.33	28.87	83.89	18.93	10.39	15.90	39.00	7.16	2.84
G16	16.23	3.89	32.87	80.00	18.31	14.78	14.74	33.93	6.45	3.33
G17	19.09	5.67	27.82	80.33	16.53	14.89	16.40	38.67	7.54	2.81
G18	18.06	5.11	34.77	60.11	18.25	13.67	15.02	27.86	7.37	2.65
G19	10.77	3.44	19.23	63.44	11.81	9.83	14.26	15.21	5.03	1.79
G20	15.32	5.11	29.32	83.33	18.25	16.56	16.83	23.03	7.08	2.80
G21	15.79	7.11	32.42	84.22	18.42	13.67	17.56	25.66	7.19	2.85
G22	22.27	8.33	43.23	98.29	20.86	17.58	18.57	56.48	7.81	4.39
G23	18.48	8.67	28.68	80.33	19.42	11.00	15.38	36.18	6.29	2.64
G24	12.06	3.11	19.18	65.33	12.48	9.89	14.22	15.38	4.69	1.82
G25	17.34	5.78	34.42	81.22	18.81	12.67	15.54	23.50	6.65	2.67
G26	15.43	4.67	24.33	82.56	18.97	14.13	17.42	30.46	6.63	2.57
G27	19.56	7.00	24.80	78.00	20.42	14.00	14.41	39.22	6.44	2.53
G28	13.16	3.67	19.09	64.78	12.69	9.78	14.20	14.91	5.28	1.84
G29	14.06	6.89	25.81	77.11	18.69	13.34	14.77	33.50	6.06	3.29
G30	14.69	7.89	29.01	74.78	20.31	11.44	16.20	39.47	6.57	3.08

CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelets per Spike), PL (Peduncle Length), SL (Spike Length), BGW (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight)

Number of Tillers per Plant (NTP) showed strong positive correlations with FLA ($r = 0.65$), Plant Height (PH) ($r = 0.66$), and Spikelets per Spike (SPS) ($r = 0.64$), indicating that genotypes with more tillers tend to have larger flag leaves, taller plants,

and increased spike fertility. This highlights the role of NTP in overall plant vigor and yield potential. Flag Leaf Area (FLA) correlated significantly with Main Spike Weight (MSW) ($r = 0.73$), indicating that a larger flag leaf contributes to increased spike weight, which directly influences grain production. Its correlation with PH ($r = 0.58$) and SL ($r = 0.52$) further supports its importance in plant growth and reproductive efficiency. Plant Height (PH) exhibited strong correlations with Spike Length (SL) ($r = 0.70$) and Peduncle Length (PL) ($r = 0.69$), suggesting that taller plants tend to have longer spikes and peduncles, which may enhance grain yield. Its moderate correlation with GYS ($r = 0.44$) indicates that plant height can contribute to grain yield but is not the sole determining factor (Khan et al., 2021; Moualeu-Ngangué et al., 2020).

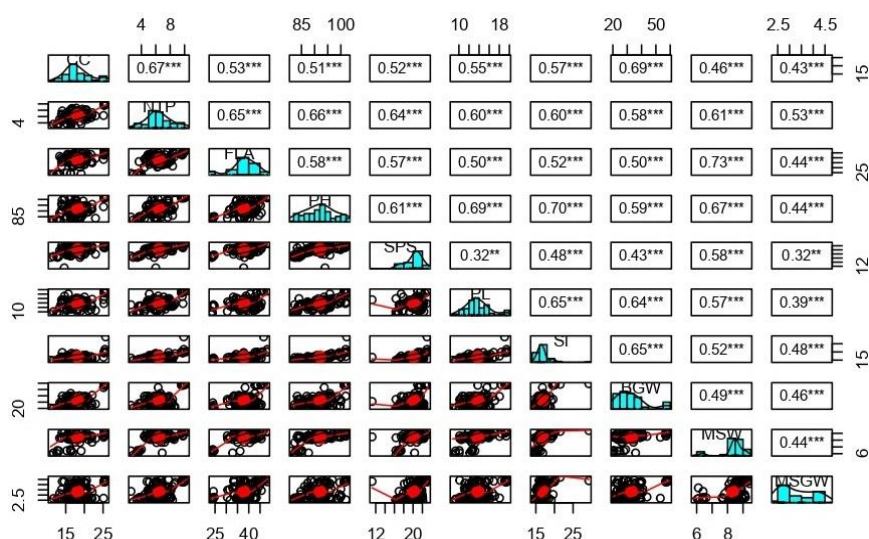


Figure 1. Histogram graph of correlation among studied traits in normal environment. CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelet per Spike), PL (Peduncle Length), SL (Spike Length), BY (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight)

Spikelets per Spike (SPS) showed positive associations with key yield components, particularly MSW ($r = 0.58$), reinforcing the role of spikelet number in determining final grain output. However, its relatively weaker correlation with MSGW ($r = 0.32$) suggests that factors beyond spikelet count influence overall grain yield. Peduncle Length (PL) and Spike Length (SL) were positively correlated ($r = 0.65$), indicating that genotypes with longer peduncles generally have longer spikes. Their significant associations with BY (PL: $r = 0.64$, SL: $r = 0.65$) emphasize their contribution to biomass accumulation and overall plant productivity. Biological Yield (BY) exhibited a strong positive correlation with CC ($r = 0.69$) and PL ($r = 0.64$), confirming that enhanced biomass production is associated with greater photosynthetic efficiency and plant vigor. However, its moderate correlation with MSGW ($r = 0.46$) suggests that while biomass is crucial, grain yield also depends on other physiological and genetic factors (Kebede et al., 2019). Main Spike Weight (MSW) showed the highest correlation with FLA ($r = 0.73$), highlighting the importance of flag leaf size in spike development and grain filling. Its positive association with SPS ($r = 0.58$) and SL ($r = 0.52$) indicates that both spike structure and weight significantly influence final grain yield. Main Spike Grain Weight (MSGW), the ultimate economic trait, was

positively correlated with all traits, particularly SL ($r = 0.48$), NTP ($r = 0.53$), and MSW ($r = 0.44$). These relationships suggest that genotypes with longer spikes, more tillers, and heavier spikes are likely to produce higher grain yields under normal conditions (Desta et al., 2024; Moualeu-Ngangué et al., 2020).

The Pearson's correlation analysis of barley genotypes under normal conditions highlighted several significant interrelationships between agronomic and yield-related traits that can inform breeding strategies. Chlorophyll content (CC) showed positive correlations with number of tillers per plant (NTP) ($r = 0.67$), biological yield (BY) ($r = 0.69$), and flag leaf area (FLA) ($r = 0.53$), suggesting that higher chlorophyll content promotes better tillering and biomass production. NTP also had strong positive correlations with FLA ($r = 0.65$), plant height (PH) ($r = 0.66$), and spikelets per spike (SPS) ($r = 0.64$), indicating that genotypes with more tillers tend to have taller plants, larger leaves, and more fertile spikes. Furthermore, FLA showed a strong positive correlation with main spike weight (MSW) ($r = 0.73$), and MSW itself was positively associated with main spike grain weight (MSGW) ($r = 0.44$). These relationships suggest that traits such as FLA, NTP, and MSW are critical for improving grain yield potential under normal conditions. Similar results with respect to maturity time and plant height in barley have also been reported in other research (Benlioglu et al., 2024; Khan et al., 2021).

The strong correlations observed among key agronomic traits suggest that traits such as NTP, FLA, PH, MSW, and SL can serve as effective selection criteria for improving grain yield in barley breeding programs. Particularly, FLA and MSW stand out as crucial determinants of grain productivity, while CC and BY highlight the role of photosynthetic efficiency in overall plant performance. These relationships provide valuable insights for selecting high-yielding genotypes under normal growing conditions (Benlioglu et al., 2024).

Correlation under drought conditions

The Pearson's correlation analysis among different quantitative traits of barley genotypes under drought conditions provides insights into how these traits interact under water-limited environments (*Fig. 2*). Understanding these correlations, helps identify key traits that contribute to drought tolerance and yield stability, guiding effective breeding strategies. Chlorophyll Content (CC) exhibited significant positive correlations with most agronomic and yield-related traits, including Flag Leaf Area (FLA) ($r = 0.65$), Biological Yield (BGW) ($r = 0.59$), and Spike Length (SL) ($r = 0.64$). These relationships indicate that higher chlorophyll content contributes to sustained photosynthesis and biomass production even under drought stress. Additionally, its correlation with Main Spike Grain Weight (MSGW) ($r = 0.56$) suggests that maintaining chlorophyll stability is vital for yield resilience. Previously results identified yield-related traits, particularly chlorophyll content positively correlated with yield related in barley genotypes under drought conditions, can guide breeding strategies by selecting for these traits to enhance drought resistance and improve overall yield stability in barley varieties (Jouyban et al., 2015; Saidi et al., 2024).

Number of Tillers per Plant (NTP) was positively correlated with Biological Yield (BGW) ($r = 0.72$), suggesting that genotypes with more tillers tend to have higher biomass accumulation. It also showed moderate correlations with SPS ($r = 0.62$) and MSW ($r = 0.51$), highlighting its role in maintaining productivity under drought conditions. Flag Leaf Area (FLA) showed strong associations with MSW ($r = 0.64$) and

BGW ($r = 0.65$), reinforcing its role in biomass accumulation and spike weight. Its correlation with Plant Height (PH) ($r = 0.67$) and Spike Length (SL) ($r = 0.59$) indicates that genotypes with larger flag leaves tend to have taller plants and longer spikes, which may enhance drought adaptation by increasing water-use efficiency (Varshney et al., 2012; Subhani et al., 2015; Saidi et al., 2024). Plant Height (PH) was strongly correlated with SL ($r = 0.71$) and FLA ($r = 0.67$), indicating that taller plants under drought conditions maintain better flag leaf development and longer spikes, which can contribute to reproductive success. Its correlation with MSGW ($r = 0.53$) suggests that while taller plants may have a yield advantage under stress, other factors also play a role. Spikelets per Spike (SPS) exhibited significant correlations with NTP ($r = 0.62$) and FLA ($r = 0.61$), suggesting that genotypes with more tillers and larger flag leaves tend to have a higher number of spikelets per spike, which may support grain retention under drought. Its moderate association with MSGW ($r = 0.48$) suggests that increased spikelet number alone does not guarantee high grain yield under stress (Desta et al., 2024; Mohi-Ud-Din et al., 2024).

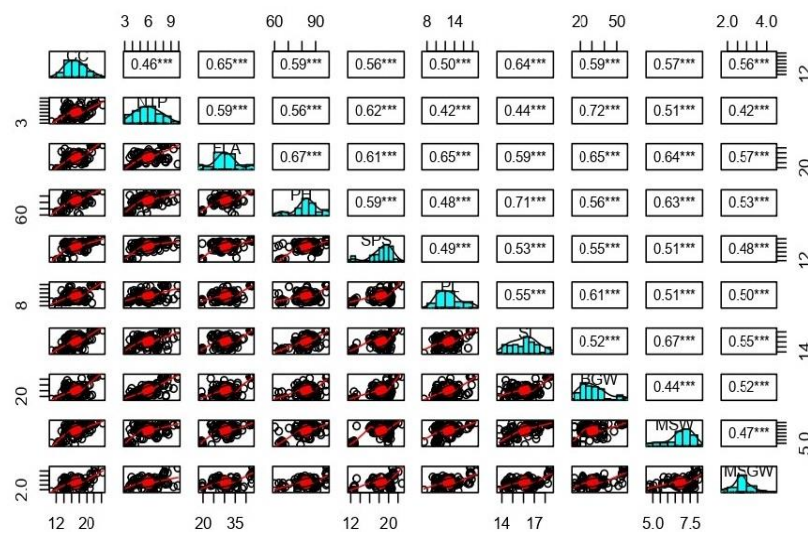


Figure 2. Histogram graph of correlation among studied traits under drought environment. CC (Chlorophyll Content), NTP (Number of Tillers per Plant), FLA (Flag Leaf Area), PH (Plant Height), SPS (Spikelet per Spike), PL (Peduncle Length), SL (Spike Length), BY (Biological weight per plant), MSW (Main Spike Weight), MSGW (Main spike Grain weight)

Peduncle Length (PL) and Spike Length (SL) were strongly correlated ($r = 0.55$), implying that longer peduncles contribute to spike elongation, which may be beneficial for grain development under drought. Their correlations with BGW (PL: $r = 0.61$, SL: $r = 0.52$) highlight their contribution to biomass accumulation. Biological Yield (BGW) was highly correlated with NTP ($r = 0.72$), indicating that increased tiller production contributes to higher biomass even under drought stress (Hejazi and Panahi, 2025; Varshney et al., 2012). Its positive association with MSW ($r = 0.44$) and MSGW ($r = 0.52$) suggests that while biomass accumulation is important, efficient resource partitioning toward grain production is necessary for improved yield. Main Spike Weight (MSW) showed a strong correlation with SL ($r = 0.67$) and FLA ($r = 0.64$), indicating that spike weight is influenced by both flag leaf size and spike length. Its moderate correlation with MSGW ($r = 0.47$) suggests that heavier spikes contribute to

grain yield under drought, but other factors such as grain filling efficiency play a role (Istanbuli et al., 2020; Khalili et al., 2016).

Main Spike Grain Weight (GYS), the ultimate indicator of productivity, was positively correlated with FLA ($r = 0.57$), SL ($r = 0.55$), and BGW ($r = 0.52$). These relationships suggest that flag leaf size, spike length, and biomass contribute to maintaining grain yield under drought conditions. However, its relatively moderate correlations with most traits imply that multiple physiological and genetic mechanisms interact to determine yield stability under stress (Hebbache et al., 2024).

Under drought conditions, key traits such as NTP, FLA, and spike length (SL) remained strongly correlated with grain yield components. CC showed significant positive correlations with FLA ($r = 0.65$), biological yield (BGW) ($r = 0.59$), and SL ($r = 0.64$), indicating that maintaining chlorophyll stability helps sustain biomass and photosynthesis under stress. NTP had a strong correlation with BGW ($r = 0.72$), emphasizing the role of tillering in biomass accumulation under drought. FLA's strong correlations with MSW ($r = 0.64$) and BGW ($r = 0.65$) further highlight its importance in biomass production and reproductive success under stress. Additionally, plant height (PH) showed strong correlations with SL ($r = 0.71$) and FLA ($r = 0.67$), suggesting that taller plants with larger flag leaves and longer spikes are more resilient to drought (Findurová et al., 2023; Jouyban et al., 2015; Mohi-Ud-Din et al., 2024). The correlations of MSW with SL ($r = 0.67$) and FLA ($r = 0.64$) indicate that spike weight and grain yield under drought are influenced by these traits, while main spike grain weight (MSGW) had moderate correlations with BGW ($r = 0.52$) and FLA ($r = 0.57$), emphasizing the importance of biomass and leaf size in sustaining yield. These findings suggest that breeding for enhanced tillering, flag leaf size, and spike length could improve drought tolerance and yield stability in barley (Benlioglu et al., 2024; Elakhdar et al., 2022).

Under drought stress, traits such as NTP, FLA, SL, and MSW play a crucial role in maintaining yield potential. FLA and SL emerge as key contributors to MSW and MSGW, highlighting their importance in spike development and grain production. Additionally, BGW and NTP exhibit strong correlations, suggesting that biomass accumulation and tiller production are critical for drought adaptation (Benlioglu et al., 2024; Hejazi and Panahi, 2025). These findings suggest that breeding strategies targeting genotypes with enhanced flag leaf size, spike length, and efficient biomass utilization can improve drought tolerance and yield stability in barley (Khan et al., 2021; Slawin et al., 2024).

Conclusion

This study highlights the significant impact of drought stress on trait correlations in barley genotypes, revealing notable genetic variability and genotype-by-environment interactions. The reductions in Chlorophyll Content (CC), Flag Leaf Area (FLA), Plant Height (PH), and Biological Yield (BY) under drought conditions indicate the adverse effects of water stress on plant physiology and productivity. Specifically, CC declined from 18.21 to 17.15, FLA from 37.81 cm² to 30.51 cm², PH from 92.07 cm to 82.95 cm, and BY from 33.03 g to 30.61 g, highlighting the sensitivity of these traits to drought. However, the stability of traits like Number of Tillers per Plant (NTP) and Spike Length (SL) suggests their importance in breeding for drought resilience. Correlation analysis reinforced these findings, showing strong associations such as CC with BY ($r = 0.69$)

and SL ($r = 0.64$), and NTP with PH ($r = 0.66$) and BGW ($r = 0.72$), emphasizing the role of biomass accumulation and efficient photosynthesis in sustaining grain yield under drought conditions. This study underscores the necessity of integrating trait correlations into multi-trait selection approaches to improve breeding strategies for drought tolerance. Future breeding efforts should prioritize genotypes with superior biomass production, spike fertility, and grain weight retention to optimize barley performance under water-limited conditions. Incorporating these insights into selection programs will facilitate the development of high-yielding, drought-resilient barley cultivars, ensuring sustainable productivity in drought-prone regions.

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Data availability. All relevant data are included in the manuscript.

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