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Integrating Quality Traits and Stomatal Adaptations for Improving Drought Tolerance in Barley

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ABSTRACT

Drought stress is a major constraint limiting barley productivity and quality under changing climatic conditions, necessitating the development of resilient genotypes. This study aimed to characterize genetic variability in quality traits and stomatal adaptations associated with drought tolerance using a line × tester mating design. Eight barley lines were crossed with four testers to produce 32 F_1 crosses, which were evaluated under normal and drought-stressed environments. Significant variation was observed among genotypes, parents, and crosses for all studied traits, with line × tester interactions being particularly significant under drought conditions, indicating the involvement of both additive and non-additive gene action. Cell membrane stability (CMS), protein content, gluten content, stomatal size, stomatal frequency, and leaf venation exhibited considerable variability, reflecting strong genetic potential for drought adaptation. Crosses generally outperformed parents, with combinations such as (L3 × T2), (L6 × T2), and (L1 × T1) showing superior performance under stress. Line L3 and testers T2 and T3 were identified as strong general combiners, contributing positively to multiple traits. Stomatal traits demonstrated a coordinated response under drought, where optimized stomatal size and increased frequency improved water-use efficiency, while higher cell membrane stability reflected enhanced cellular integrity. The predominance of non-additive gene action for several traits suggests the importance of superior cross combinations and delayed selection in advanced generations. Overall, the findings highlight the potential of integrating physiological and quality traits with combining ability analysis to develop drought-resilient barley genotypes. This study provides valuable insights for breeding programs aimed at sustainable food production under water-limited environments.

Keywords: barley, water stress, general combiner, gene action, SCA.

INTRODUCTION

Population growth and climate change are major challenges to global food security, necessitating the development of high-yielding and high-quality barley varieties. Barley breeders are therefore focused on improving grain yield by utilizing existing genetic resources to develop improved cultivars (Khalid et al., 2024; Ahmed et al., 2025a). However, increasing population pressure, low precipitation, and depletion of

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groundwater resources have significantly threatened barley production (Sultan et al., 2016). With decreased availability of both arable land and high-quality irrigation water, the need is now to develop crops that can grow in either favourable or unfavourable environments. Genetic, physiological, biochemical, and molecular advances will allow for improved crop adaptability to drought conditions (Sallam et al., 2019). Drought is one of the most critical abiotic stresses because it changes gene expression and metabolism, which then impacts plant growth and productivity. Under drought stress conditions in barley, yield has been found to be positively related to drought tolerance (Mazloumi Oskouiee et al., 2025; Sarfraz et al., 2025; Yang et al., 2025a).

Drought conditions impact the water status of plants. The effects of drought stress include decreased photosynthesis, higher rates of oxidative damage, and decreased growth. These issues lead to significant reductions in yield. The common signs of dry conditions include reduction in leaf area, reduction in the number of tillers, reduced grain weight, and an overall reduction in productivity (Ahmed et al., 2014; Sallam et al., 2019; Ahmad et al., 2025). Therefore, developing cultivars with mechanisms to tolerate or avoid water stress is essential. This requires a deeper understanding of genetic variability, combining ability, and plant responses to drought (Cai et al., 2020). Crop yields are severely affected by drought globally, with barley being a prime example of how drought can cause loss of growth and yield as well as poor grain quality. Under water-deficit conditions, crop physiological process including photosynthesis, nutrient uptake and reproduction are negatively impacted (Liu et al., 2017). Water-limiting conditions influence the quality traits of grain, including starch composition and protein levels. Physiological parameters, such as cell membrane stability, chlorophyll content, and stomatal conductance have significant influence on plant performance under drought stress (Bănică et al., 2008; Petcu et al., 2014; Rahim et al., 2021; Ahmed et al., 2025b).

Earlier investigations have shown that traits such as higher cell membrane stability and chlorophyll concentration have an effect on increased drought tolerance by enabling plants to sustain their cell structure and to be efficient photosynthetically (Ahmed et al., 2025a; Sutthachai et al., 2025). Stomatal morphology (size and density) is among the most critical traits that determine the amount of water lost through transpiration and the amount of gas exchanged; thus, indirectly determining the plant's water-use efficiency. Genotypes with optimal stomatal size and density will therefore demonstrate the best drought tolerance (Cai et al., 2020). Genetic mechanisms controlling these traits are often complex (including non-additive gene effects). Therefore, there is a need for progressive breeding strategies that combine conventional and genomic techniques (Ahmed et al., 2014). Barley has a high degree of genetic diversity, creating an excellent opportunity for developing drought-resistant cultivars. Drought-resistant plant characteristics include the following: (1) cell membrane stability; (2) using leaves to capture sunlight; (3) control of leaf and plant surface temperatures; and (4) how stomata regulate evaporative cooling through their behaviour. For the best parent selection for breeding programs, knowledge of general and specific combining ability, as well as gene interactions, is necessary (Sultan et al., 2016; Yang et al., 2025b). Line × tester analysis remains a valuable tool for evaluating parental performance and identifying superior crosses. Integrating physiological traits with combining ability analysis enhances the selection of genotypes adapted to water-limited environments (Kalyar et al., 2024).

Considering more frequent instances of severe impact from climate change and increasing levels of lack of rainfall (scarcity of water) developing drought resistant barley varieties will be necessary. This research provides an understanding of barley tolerance to drought through the combination of genetic data and essential morphologic and physiologic characteristics. This information will be helpful to breeders and assist with the development of more drought tolerant cultivars that can maintain production levels even with limited water.

MATERIAL AND METHODS

A total of 44 barley genotypes (8 lines, 4 testers, and 32 F_1 crosses) were evaluated under normal and drought-stressed conditions using a Randomized Complete Block Design with three replications at the Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur.

The genotypes were selected based on their potential for drought tolerance and overall performance. A line × tester mating design was used, where each female line was crossed with all testers to generate diverse genetic combinations. Seeds were sown at 6-inch plant spacing and 12-inch row spacing, and after emergence, seedlings were thinned to five plants per genotype to minimize competition and ensure uniform growth. Two irrigation regimes were maintained: well-watered conditions (100% field capacity) and drought stress imposed by withholding irrigation at the tillering stage. All other agronomic practices were kept uniform across treatments. Data were recorded at maturity from five representative plants per genotype under both environments to ensure reliable assessment of the studied traits.

Traits Measurement and Statistical Analysis

Cell membrane stability was estimated using standard equations (Blum and Ebercon, 1981). Grain quality traits, including protein and gluten contents, were determined following AACC standard procedures (Committee, 2000). Stomatal frequency, stomatal size and leaf venation were measured using established microscopic methods after treating leaf samples with Carnoy's solution (Sheehan and Hrapchak, 1973). Data were analyzed using ANOVA to test significance among genotypes and treatments (Steel and Torrie, 1981), and traits showing variability were further evaluated through line × tester analysis to estimate combining ability (Kempthorne, 1957).

RESULTS AND DISCUSSION

Analysis of variance showed significant differences among genotypes, parents, and crosses under both environments, while line × tester interaction was significant under drought (Table 1), indicating the role of both additive and non-additive gene action as reported by the researchers in barely (Kalyar et al., 2024).

Table 1. L×T Genetic analysis of variances for studied traits under normal and drought environment

Traits	REP	Genotypes	Parents	Crosses	P.vs C	Lines	Testers	L × T	Error
CMS-N	12.64*	73.29*	72.45*	9.78*	20.5*	6.19*	13.33*	10.47*	1.74
CMS-D	10.92*	40.75*	59.16*	3.30*	99.01*	3.15*	8.89*	2.55*	0.08
PP-N	14.02*	11.91*	5.06*	6.80*	24.55*	7.14*	1.76*	7.41*	0.01
PP-D	12.90*	5.87*	6.15*	1.30*	14.60*	1.16*	2.10*	1.23*	0.01
GC-N	15.86*	46.53*	22.61*	5.50*	158.2*	4.60*	3.35*	6.11*	1.06
GC-D	13.62*	25.61*	21.28*	0.92*	83.51*	0.51*	0.42*	1.13*	0.03
SS-N	40.84*	7.93*	7.29*	3.64*	14.13*	2.58*	2.66*	4.13*	0.03
SS-D	38.66*	4.38*	4.89*	0.28*	1.26*	0.09	0.01ns	0.38*	0.14
SF-N	28.6*	4.44*	2.34*	1.81*	109*	2.02*	1.58 ns	1.78*	0.95*
SF-D	32.46*	7.67*	6.04*	1.20*	22.1*	1.51*	0.46 ns	1.21*	0.2
LV-N	0.07 ns	0.60*	0.79*	0.20*	10.80*	0.33*	0.25 ns	0.16 ns	0.11
LV-D	0.83*	1.06*	0.9*	0.35*	24.71*	0.35*	0.15ns	0.38*	0.11

(CMS) Cell Membrane Stability, (PP) Protein %, (GC) Gluten Content, (SS) Stomata Size, (SF) Stomata Frequency, (LV) Leaf venation.

Table 2. Mean performance of parents under normal and drought environments

Traits	L1	L2	L3	L4	L5	L6	L7	L8	T1	T2	T3	T4
CMS-N	57.5	56.4	58.5	53.5	54.4	53.5	55.4	52.1	50.3	51.2	47.2	40.2
CMS-D	54.2	53.3	55.8	51.7	50.1	47.2	53.4	52.4	45.8	46.6	46.4	40.3
PP-N	14.7	14.9	15.4	13	15.2	16.9	16.9	15.1	13.6	14.1	12.7	11.1
PP-D	13.3	12.3	16.4	14.4	13.8	15.7	14.7	15.7	14.3	15.3	12.3	13.3
GC-N	23.3	25.3	27.3	24.3	23.1	21.3	24.2	22.2	19.3	20.3	18.3	19.3
GC-D	24.6	27.2	29.2	26.2	25.2	23.2	24.2	24.2	20.8	21.8	20.6	21.5
SS-N	0.36	0.35	0.36	0.35	0.34	0.03	0.33	0.33	0.32	0.33	0.32	0.31
SS-D	0.31	0.32	0.33	0.31	0.34	0.34	0.32	0.32	0.3	0.31	0.3	0.3
SF-N	12.3	14.2	14.4	12.9	14.9	12.8	12.9	12.8	12.1	12.8	12.5	12.7
SF-D	12.0	14.6	14.4	11.9	14.9	11.9	13.9	12.4	11.1	11.7	11.5	11.3
LV-N	8.82	8.24	8.93	8.55	8.46	8.76	8.33	8.54	7.81	7.93	7.44	7.42
LV-D	8.4	8.21	8.45	8.21	8.11	7.91	8.35	7.5	7.04	7.51	7.15	7.02

Cell Membrane Stability (CMS)

Cell membrane stability (CMS) is a key physiological indicator of drought tolerance, reflecting the ability of plant cells to maintain membrane integrity under stress conditions (Ahmed et al., 2014; Ardabili and Shahbazi, 2015). In the present study, CMS showed wide variation among genotypes, indicating substantial genetic diversity for this trait. Under normal conditions, CMS ranged from 40.2% (T4) to 58.5% (L3), with L3 showing the highest performance among parents. Under drought, values decreased slightly, ranging from 40.3% (T4) to 55.8% (L3), indicating stress-induced reduction. Overall, lines performed better than testers under both environments (Table 2). Under normal conditions, line L3 (1.23*) showed the highest positive GCA effect, followed by tester T3 (0.86*), while L6 and L1 showed significant negative effects. Under drought, L4 (0.83*) and L3 (0.58*) remained strong positive combiners, whereas L6 and T3 showed negative effects. Most other lines exhibited non-significant effects, indicating limited contribution (Table 3).

Under normal conditions, CMS ranged from 58.88% (L6 × T1) to 65.54% (L3 × T2) as displayed in Figure 1A. Under drought, values slightly decreased, ranging from 54.52% (L7 × T1) to 58.67% (L3 × T2) as mentioned in Figure 1B. Cross (L3 × T2) consistently exhibited superior performance across environments. Under normal conditions (Figure 2A), SCA effects for CMS ranged from -2.86 in (L2 × T3) to 3.48 in (L3 × T2), indicating considerable variation among crosses. Under drought (Figure 2B), values ranged from -1.16 in (L5 × T4) to 1.78 in (L3 × T2). Cross (L3 × T2) showed the best SCA performance in both environments. From a physiological perspective, higher CMS under drought conditions reflects reduced membrane damage, lower electrolyte leakage, and better maintenance of cellular homeostasis. Similar observations have been reported by (Ardabili and Shahbazi, 2015; Ahmed et al., 2025a), who demonstrated that improved membrane stability is closely associated with enhanced stress tolerance and better yield performance under drought conditions (Ahmed et al., 2014).

Additionally, our findings provide further evidence for utilizing CMS as a reliable indicator of drought tolerance whereby high CMS values indicate that the associated genotypes and crosses are more likely to maintain metabolic activity and grow during periods of stress than genotypes/crosses with lower CMS values (Ahmed et al., 2021; Mazloumi Oskouiee et al., 2025). Thus, when breeding to develop drought-tolerant barley genotypes with enhanced adaptability and performance in environments with limited water, CMS should be appropriately applied as a selection criterion (Ardabili and Shahbazi, 2015; Ahmed et al., 2025a).

Protein Content

Protein content showed significant variation across genotypes and environmental conditions, indicating its sensitivity to both genetic factors and drought stress. Under normal conditions, protein content ranged

from 11.1% (T4) to 16.9% (L6 and L7), with lines generally outperforming testers. Under drought, values ranged from 12.3% (L2 and T3) to 16.4% (L3), showing an increase in some genotypes (Table 2). This suggests differential response of protein accumulation under stress. Under normal conditions, L7 (1.02 ns) and L3 (0.77*) showed relatively higher GCA effects, while L6 (-0.53*) and T4 (-0.52*) had significant negative effects. Under drought, L3 (0.27*) and T2 (0.15*) performed as better combiners, whereas T4 (-0.48*) and T3 (-0.15*) showed negative effects. Most genotypes exhibited moderate to non-significant variation as described in Table 3.

Under normal conditions, protein content ranged from 15.67% (L8 × T2) to 17.86% (L3 × T2) as depicted in Figure 1A, while under drought it increased from 15.30% to 20.37% (L3 × T2) as shown in Figure 1B. Several crosses maintained higher protein under stress, indicating enhanced nitrogen accumulation. Under normal conditions (Figure 2A), SCA effects for protein content varied from -2.44 in (L2 × T2) to 2.65 in (L3 × T2). Under drought (Figure 2B), the range was from -0.93 in (L5 × T4) to 1.41 in (L8 × T1), showing moderate variation. Crosses such as (L3 × T2), (L5 × T2), and (L8 × T1) showed favourable SCA effects.

The increase in protein content under drought conditions can be attributed to reduced carbohydrate accumulation, particularly starch, which leads to a relative concentration of nitrogenous compounds in the grain. This shift in grain composition reflects changes in nitrogen metabolism and assimilate partitioning under stress. Furthermore, several crosses showed significant specific combining ability (SCA) effects, suggesting that non-additive genetic interactions also contribute to enhanced performance.

These results are in agreement with previous studies showing that there are strong genotype × environment interactions regarding protein content (Panwar and Sharma, 2019). Current results show that protein levels increase in drought-stressed plants due to limited availability of carbohydrates for storage (Cai et al., 2013) but this protein accumulation is usually achieved at the expense of reduced crop yield; thus, there is a physiological trade-off. Therefore, selection strategies should be developed to achieve optimal balance between yield and protein under drought conditions (Van Landschoot, 2011; Yu et al., 2017). Crosses such as L3 × T2 have good potential for improving protein levels in drought environments.

Gluten Content

Gluten content also exhibited considerable variation among genotypes and environments, reflecting its dependence on both genetic background and environmental conditions. Under normal conditions, gluten content varied from 18.3% (T3) to 27.3% (L3), while under drought it increased to a range of 20.6% (T3) to 29.2% (L3). Lines consistently showed higher values than testers. The overall increase under drought indicates enhanced protein concentration due to stress conditions (Table 2). Under normal conditions, L3 (0.90*) and T2 (0.48*) exhibited strong positive GCA effects, while L6 (-0.69*) and T4 (-0.65*) showed significant negative effects. Under drought, L3 (0.31*) and T2 (0.19*) remained superior combiners (Table 3). Most other lines showed non-significant or minor effects.

Under normal conditions, gluten content ranged from 28.33% to 33.33%, with the highest value recorded in (L3 × T2) as shown in Figure 1A. Under drought, values ranged from 28.87% (L3 × T1) to 30.85% (L3 × T2) exhibited in Figure 1B. Crosses generally maintained stable gluten content under stress, with slight reductions or improvements depending on genotype. For gluten content under normal conditions (Figure 2A), SCA values ranged from -1.73 in (L4 × T1) to 2.35 in (L3 × T2). Under drought, values varied from -0.78 in (L6 × T2) to 0.99 in (L3 × T2). Cross (L3 × T2) consistently exhibited the highest positive SCA effect across both environments (Figure 2B).

The observed increase in gluten content under drought stress may be associated with enhanced accumulation of storage proteins,

particularly gliadins and glutenins, due to reduced starch synthesis (Meng et al., 2023; Ramadan et al., 2026). Additionally, several crosses showed significant specific combining ability (SCA) effects (Figure 2), suggesting that non-additive gene action plays an important role in improving gluten content.

These findings are in agreement with previous studies reporting significant variability in gluten content under drought stress (Yu et al., 2017). Our findings lend credence to the theory that environmental stress helps improve grain quality traits, due to changes in metabolic pathways. Similar to the relationship between yield and protein content, improving gluten may be at the expense of potentially lowering yields, highlighting the necessity to apply balanced selection criteria (Mazloumi Oskouiee et al., 2025). Overall, these findings illustrate that gluten levels are affected by both additive and non-additive genetic influences on the level of gluten produced through specific hybrids, particularly with (L3 × T2), in improving grain quality under drought conditions while providing continued adaptability.

Table 3. General combining ability effects of parents under normal and drought environment

Lines & Testers	Environments	CMS	PP	GC	SS	SF	LV
L1	Normal	-0.02*	0.52*	0.65*	-1.53*	0.35*	0.02*
	Drought	-0.05*	0.48*	-0.16*	1.53*	-0.41*	-0.01*
L2	Normal	-0.02ns	-0.48ns	-0.86ns	30.97ns	-0.22 ns	0.02ns
	Drought	0.12 ns	-0.45 ns	0.03ns	0.53ns	-0.12ns	0.16ns
L3	Normal	1.23*	0.77*	0.9*	60.97*	0.83*	0.17*
	Drought	0.58*	0.27*	0.31*	5.03*	-0.31*	0.18*
L4	Normal	-0.02*	0.27*	-0.35*	-75.98*	-0.29*	0.07*
	Drought	0.83*	0.01*	-0.1*	-17.22*	0.59*	0.13*
L5	Normal	-1.15ns	-0.28 ns	0.15 ns	38.47 ns	-0.25 ns	0.05 ns
	Drought	-0.75 ns	-0.05 ns	-0.28 ns	-3.72ns	-0.23 ns	0.02 ns
L6	Normal	-0.18*	-0.53*	-0.69*	-19.03*	-0.08*	0.05*
	Drought	-0.26*	0.18*	0.12*	14.28*	-0.02*	-0.34*
L7	Normal	-0.52ns	1.02 ns	-0.1 ns	-49.86 ns	-0.4 ns	0.01 ns
	Drought	-0.31 ns	-0.09 ns	-0.14 ns	1.28ns	0.04 ns	-0.11 ns
L8	Normal	0.68ns	-1.27 ns	0.32 ns	15.97 ns	0.05 ns	-0.39 ns
	Drought	-0.15 ns	-0.35 ns	0.22 ns	-1.72ns	0.44 ns	-0.01 ns
T1	Normal	-0.75ns	0.24n ns	0.23 ns	20.97 ns	0.14 ns	-0.05 ns
	Drought	-0.73 ns	-0.33ns	-0.05 ns	-0.47ns	-0.06 ns	-0.04 ns
T2	Normal	0.37*	0.13*	0.48*	21.81*	0.05*	-0.11*
	Drought	0.22*	0.15*	0.19*	0.53*	0.2*	-0.07*
T3	Normal	0.86*	0.01*	-0.1*	-48.75*	0.19*	0.11*
	Drought	-0.22*	-0.15*	-0.03*	1.78*	-0.02*	0.01*
T4	Normal	0.02*	-0.52*	-0.65*	1.53*	-0.35*	-0.02*
	Drought	0.05*	-0.48*	0.16v	-1.53*	0.41*	0.01*

Stomatal Size (SS)

Stomatal size is an important anatomical trait that influences gas exchange capacity and transpiration efficiency, particularly under drought stress conditions (Lv et al., 2023). Values for stomatal size (SS) were scaled (÷10,000) for better presentation without affecting statistical interpretation. Among parents, under normal conditions, stomatal size ranged from 0.31 μm² (scaled $\times 10^{-4}$) (T4) to 0.36 μm² (scaled $\times 10^{-4}$) (L1 and L3), while under drought it decreased to 0.30–0.34 μm² (scaled $\times 10^{-4}$) as mentioned in Table 2. This reduction reflects an adaptive response to minimize water loss under stress. Lines generally maintained slightly higher values compared to testers. Under normal conditions, L3 (60.97*) and T2 (21.81*) showed strong positive GCA effects, while L4 (-75.98*) and T3 (-48.75*) exhibited significant negative effects. Under drought, L6 (14.28*) and L3 (5.03*) showed positive effects, whereas L4 (-17.22*) and T4 (-1.53*) remained negative combiners. Several

genotypes showed non-significant responses (Table 3).

Under normal conditions (Figure 1A), stomatal size ranged from 0.33 µm² (scaled $\times 10^{-4}$) (L4 × T3) to 0.38 µm² (scaled $\times 10^{-4}$) (L3 × T2 and L4 × T2). Under drought (Figure 1B), values were slightly reduced and stabilized around 0.34-0.35 µm² (scaled $\times 10^{-4}$). Most crosses maintained consistent stomatal size under stress, indicating structural stability. Under normal conditions (Figure 2A), SCA effects for stomatal size ranged from -178.47 in (L8 × T4) to 207.64 in (L3 × T2), indicating strong variability among crosses. Under drought, the range was from -51.03 in (L1 × T2) to 64.47 in (L3 × T2). Cross (L3 × T2) again showed the strongest positive SCA effect under both conditions (Figure 2B). Similar findings have been reported where stomatal size is influenced by both genetic components and environmental conditions, contributing to drought adaptation (Ahmed et al., 2025b; Yang et al., 2025a). Our results are also supported by (Abdelrady et al., 2024; Yang et al., 2025b), who reported that optimized stomatal dimensions enhance water-use efficiency and improve plant performance under stress (Liu et al., 2017).

From a physiological perspective, relatively smaller or optimally sized stomata are associated with faster opening and closing dynamics, enabling plants to respond rapidly to environmental fluctuations (Rahim et al., 2021; Robertson et al., 2021). This improves water-use efficiency by reducing unnecessary water loss while maintaining sufficient CO_2 uptake. Therefore, stomatal size emerges as a critical trait for balancing transpiration and photosynthesis, ultimately contributing to improved drought tolerance in barley (Cai et al., 2020).

Stomatal Frequency (SF)

Stomatal frequency plays a crucial role in determining the balance between CO_2 uptake and water loss, thereby influencing photosynthetic efficiency and plant adaptation under drought stress (Robertson et al., 2021). Among parents, in normal conditions, stomatal frequency ranged from 12.1 mm^{-2} (T1) to 14.9 mm^{-2} (L5), while under drought it varied from 11.1 mm^{-2} (T1) to 14.9 mm^{-2} (L5). Some lines maintained or slightly increased frequency under stress, indicating variability in stomatal regulation. Testers generally showed lower values (Table 2). Under normal conditions, L3 (0.83*) and T3 (0.19*) showed significant positive GCA effects, while L4 (-0.29*) and T4 (-0.35*) showed negative effects. Under drought, L4 (0.59*) and T2 (0.20*) were positive combiners, whereas L1 (-0.41*) showed negative significant effect. Most other genotypes showed non-significant variation (Table 3).

Under normal conditions (Figure 1A), stomatal frequency ranged from 12.98 mm^{-2} (L7 × T4) to 16.40 mm^{-2} (L3 × T2). Under drought (Figure 1B), values ranged from 14.50 mm^{-2} (L6 × T1) to 16.50 mm^{-2} (L8 × T4), showing slight improvement in some crosses. Several crosses maintained or increased frequency under stress conditions. For stomatal frequency, SCA effects under normal conditions (Figure 2A) ranged from -1.42 in (L7 × T4) to 0.98 in (L1 × T4). Under drought, values ranged from -1.00 in (L6 × T1) to 0.83 in (L2 × T4) as showed in Figure 2B. Crosses such as (L1 × T4), (L7 × T1), and (L2 × T4) showed favorable SCA effects. This variability suggests a stronger influence of non-additive gene action in controlling this trait. The enhanced performance of specific crosses indicates that hybrid combinations can effectively exploit genetic interactions to improve stomatal density under stress. Similar trends have been reported by Abdelrady et al. (2024) and Ahmed et al. (2025b), where stomatal frequency was closely linked with drought adaptation and yield stability. Our findings are also in agreement with (Cai et al., 2020; Rahim et al., 2021), who demonstrated that increased stomatal frequency, when combined with optimal stomatal size, improves transpiration control and carbon assimilation under limited water conditions (Lv et al., 2023).

Furthermore, stomatal behavior under drought is regulated by physiological

mechanisms such as ABA and Ca^{2+} signaling, which enable rapid stomatal closure to minimize water loss while allowing timely reopening for photosynthesis (Liu et al., 2017; Robertson et al., 2021). This dynamic adjustment enhances water-use efficiency and helps maintain metabolic activity under stress (Moroșan et al., 2022). Overall, these findings emphasize that stomatal frequency, in combination with stomatal size, plays a significant role in drought tolerance and should be considered a key selection criterion in barley breeding programs targeting water-limited environments (Lv et al., 2023).

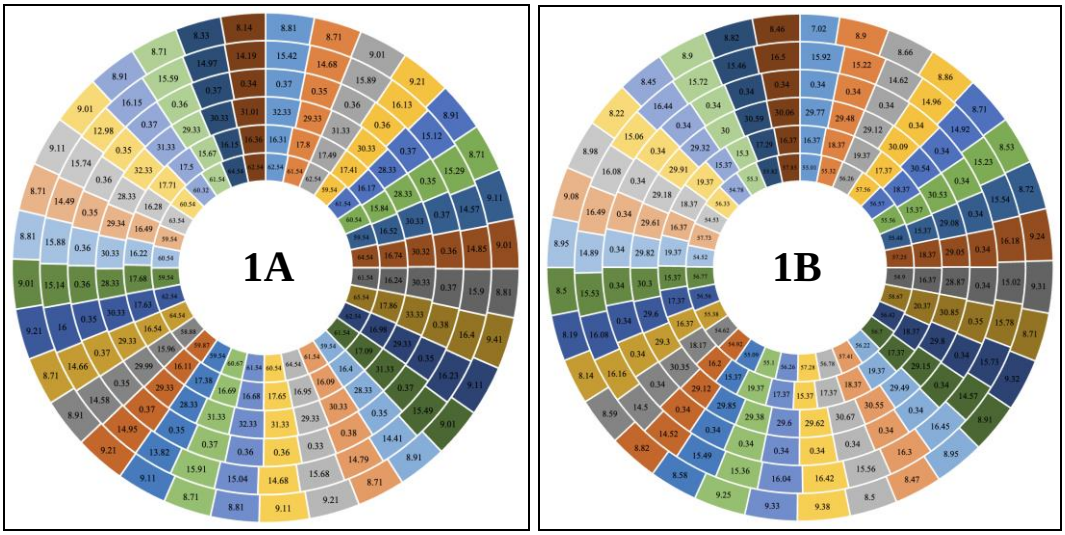

Figure 1. Performance of crosses (L × T) under normal (1A) and drought (1B) environments

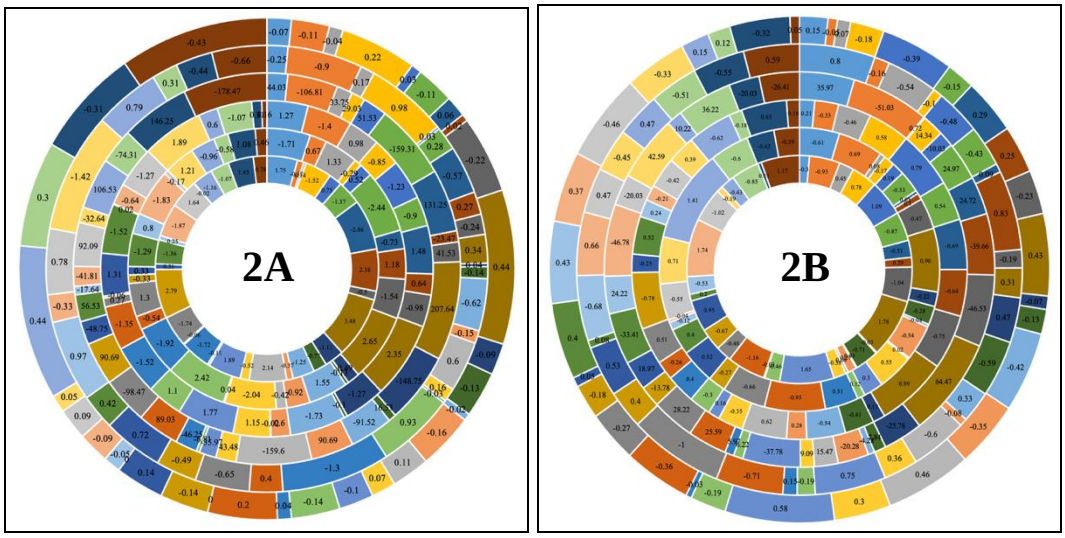

Figure 2. Specific combining ability (SCA) of F_1 crosses (L × T) under normal (2A) and drought (2B) conditions

Note: *Figure 1 presents the mean performance of cross combinations, whereas Figure 2 illustrates the specific combining ability (SCA) effects of the crosses. Both figures provide a circular visualization of trait performance of L × T crosses under normal and drought environments. The inner circle represents cell membrane stability (CMS), the second circle represents protein content (PP), the third circle represents gluten content (GC), the fourth circle represents stomatal size (SS), and the fifth circle represents stomatal frequency (SF), while the sixth (outermost) circle represents leaf venation (LV). Each color within the circles corresponds to a specific cross combination (L1 × T1 to L8 × T4), as indicated by the colored squares in the legend, and the respective values are displayed within each colored segment for the corresponding cross.*

L1 X T1	L1 X T2	L1 X T3	L1 X T4	L2 X T1	L2 X T2	L2 X T3	L2 X T4
L3 X T1	L3 X T2	L3 X T3	L3 X T4	L4 X T1	L4 X T2	L4 X T3	L4 X T4
L5 X T1	L5 X T2	L5 X T3	L5 X T4	L6 X T1	L6 X T2	L6 X T3	L6 X T4
L7 X T1	L7 X T2	L7 X T3	L7 X T4	L8 X T1	L8 X T2	L8 X T3	L8 X T4

Leaf venation (LV)

Leaf venation is a key structural trait that plays a central role in water transport, nutrient distribution, and overall plant performance under drought conditions (David et al., 2017; Panwar and Sharma, 2019). Efficient venation networks help maintain hydraulic conductivity, allowing plants to sustain physiological processes even when water availability is limited. Among parents, under normal conditions, leaf venation ranged from 7.42 mm^{-1} (T4) to 8.93 mm^{-1} (L3), while under drought it ranged from 7.02 mm^{-1} (T4) to 8.45 mm^{-1} (L3). Lines exhibited higher venation values compared to testers in both environments. The reduction under drought suggests structural adjustment to water limitation (Table 2). Under normal conditions, L3 (0.17*) and T3 (0.11*) exhibited strong positive GCA effects, while L8 (-0.39 ns) showed a negative but non-significant trend. Under drought, L3 (0.18*) and L4 (0.13*) remained strong combiners. Most lines and testers showed small or non-significant effects, indicating relatively stable genetic control (Table 3).

Under normal conditions, leaf venation ranged from 8.14 mm^{-1} (L8 × T4) to 9.41 mm^{-1} (L3 × T2) as exhibited in Figure 1A. Under drought (Figure 1B), values ranged from 7.02 mm^{-1} (L1 × T1) to 9.38 mm^{-1} (L4 × T4). Crosses such as (L4 × T4), (L3 × T3), and (L5 × T1) maintained higher venation under stress. Under normal conditions (Figure 2A), SCA effects for leaf venation ranged from -0.43 in (L8 × T4) to 0.44 in (L3 × T2) and (L8 × T1). Under drought (Figure 2B), values ranged from -0.46 in (L7 × T3) to 0.58 in (L5 × T1). Crosses (L5 × T1), (L3 × T2), and (L4 × T3) showed relatively better SCA performance for this trait. From a physiological perspective, improved venation enhances internal water movement and supports sustained photosynthetic activity under drought stress, thereby contributing to plant stability and adaptation. (Ahmed et al., 2025b; Mazloumi Oskouiee et al., 2025). However, structural modifications associated with drought tolerance may also impose certain trade-offs, such as reduced expansion or growth under limited resources (Robertson et al., 2021). Therefore, careful and balanced selection of this trait is necessary in breeding programs to ensure both stress tolerance and yield stability under fluctuating environmental conditions (Rahim et al., 2021).

CONCLUSIONS

This study provides a comprehensive evaluation of genetic variability, combining ability, and physiological mechanisms underlying drought tolerance in barley. The significant genetic variation observed among genotypes together with the strong line × tester interaction under drought stress, confirm the complex inheritance of the studied traits. The identification of line L3 and testers T2 and T3 as superior general combiners highlights their potential as key parental resources for breeding programs targeting multiple drought-adaptive traits. The superior performance of specific crosses, especially (L3 × T2) and (L6 × T2), demonstrates the importance of exploiting favorable non-additive gene effects to enhance both physiological stability and grain quality under stress conditions. The combined expression of traits such as cell membrane stability, optimized stomatal behavior, and grain protein and gluten content indicates that drought tolerance in barley is governed by an integrated network of morpho-physiological responses rather than single-trait effects. The predominance of non-additive gene action suggests that breeding strategies should emphasize selection in advanced generations to effectively capture favorable gene combinations. Future research should focus on integrating these traits with molecular markers and genomic tools to accelerate selection efficiency. Overall, this study offers a strategic framework for developing climate-resilient barley cultivars adapted to water-limited environments, contributing to sustainable crop production.

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