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Physiological and Anatomical Trait Integration Reveals Variability for Drought Resilience in Barley

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ABSTRACT

This study evaluated 20 barley (*Hordeum vulgare* L.) genotypes under well-watered and drought-induced conditions to investigate physiological and anatomical adaptations contributing to drought tolerance. Conducted using a randomized complete block design (RCBD) with three replications, the experiment quantified six key traits: Cell Membrane Thermostability (CMT), Total Chlorophyll Content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf Venation (LV), and Epidermal Cell Size (ECS). Significant genotypic ($p < 0.01$), environmental ($p < 0.05$ or $p < 0.01$), and genotype × environment interaction effects ($p < 0.05$) were observed for all traits, highlighting both genetic diversity and environmental influence on drought responses. Under drought, mean CMT and TCC decreased from 59.85 to 54.70 and from 60.11 to 50.90, respectively, while ECS increased from 2591.80 to 2689.15, indicating adaptive anatomical reconfiguration. Principal Component Analysis explained 96.42% and 97.17% of the total variation under normal and drought conditions, respectively, underscoring robust multivariate trait discrimination. Genotypes G8, G13, and G17 consistently outperformed others, exhibiting superior CMT (60.62, 60.56, 60.55), TCC (56.66, 56.43, 56.63), and ECS (2780.32 each), along with optimal stomatal and venation traits, indicating enhanced physiological resilience. Conversely, G7, G11, and G19 exhibited inferior performance across all traits. These findings affirm the utility of integrative physiological and anatomical trait analysis in identifying drought-resilient barley genotypes and provide a strong empirical basis for their incorporation into breeding programs aimed at climate adaptation and yield stability.

Keywords: barley, drought, physiology, anatomy, genotypes, resilience.

INTRODUCTION

Barley (*Hordeum vulgare* L.) has been amongst the earliest domesticated cereal crops, popular owing to its resistance to different climatic regimes, short maturity, as well as its utilization in food, animal feed, and brewing industries. Barley comes in fourth place worldwide amongst cereal crops after maize, wheat, and rice (Moualeu-Ngangué et al., 2020; Rahmati et al., 2024). Its abiotic stress-tolerating nature, specifically drought, has been a model crop in stress physiology, along with genetic stress resistance in arid-land parts of the earth along with semi-arid regions. But with enhanced climate change, traditional drought-tolerating mechanisms in barley would become ineffective, and therefore, presently there has been a need to find new sources of genetic variation along

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with adaptation traits to ensure effective crop production (Hunt et al., 2021; Al-Ashkar, 2024).

Drought ranks among the most serious abiotic stress factors impacting plant growth and productivity. It hinders plant water relations, slows down physiological activities like transpiration and photosynthetic activities, and induces oxidative stress through excessive production of reactive oxygen species (ROS). Under drought stress, at both vegetative as well as reproductive phases in cereal crops like barley, yield potential is severely compromised by disrupting factors like leaf gas exchange, water-use efficiency, and acquisition of nutrients (Kumar et al., 2024; Rahmati et al., 2024). The intricacy of drought tolerance invites a holistic approach incorporating morphological, physiological, biochemical, as well as anatomical characteristics for the recognition of genotypes tolerant to water-deficit stress (Ijaz et al., 2023; Ferioun et al., 2025).

Previously, breeding programs have rested primarily on selection using yields during stress, but to a lesser extent on physiological and anatomical characteristics of drought resistance. With the advances in plant physiology, as well as functional genomics, possibilities exist in these types of traits as effective indicators of stress tolerance. Physiological traits such as relative water content (RWC), membrane stability index (MSI), chlorophyll content, stomatal conductance, and leaf temperature are key factors influencing a genotype's ability to maintain cellular activity and gas exchange during water stress. Anatomical traits such as stomatal density, epidermal thickness, and vascular bundle adaptations have direct impacts on transpiration regulation, photosynthate partitioning, and plant structure during stress episodes (Vaezi et al., 2010; Rahmati et al., 2024).

Although physiological and anatomical responses are important, comparatively fewer investigations have combined the two aspects in a structured way to break down drought resistance in barley. Most of the existing work treats these traits separately or under controlled conditions, providing only incremental understanding of genotype performance under field drought stress (Fatemi et al., 2023; Khalid et al., 2024). Besides, there is also high genotypic variation in expression of traits so that integration of traits would enable revealing new adaptive mechanisms employed by various lines of barley. An integrated approach based on traits can hence enhance accuracy of selection of genotypes by mapping structure to performance (El-Hashash and Agwa, 2018; Kiani-Pouya et al., 2020).

Physiological characteristics like relative water content, membrane stability index, chlorophyll content, stomatal conductance, leaf temperature, and similar other characteristics were measured during sensitive stages of growth. Concomitantly, detailed anatomical studies were carried out using microtome and microscopy methods to find stomatal density, leaf and root tissue thickness, and vascular bundle characteristics. These combined data sets facilitated a simultaneous analysis of anatomical and functional responses to drought at the genotype level (Sallam et al., 2019; Rahmati et al., 2024). The integration of physiological and anatomical data not only facilitated conceptually describing drought adaptation mechanisms but also allowed selection of elite genotypes harboring a set of anatomical and functional traits (Ali et al., 2024; Kumar et al., 2024). The multi-trait genotypes are a potential resource to breeders to improve drought resistance in barley cultivars through breeding. The identification of key traits responsible for drought resistance in field conditions also provides a platform upon which marker-aided selection as well as genomics-based studies can be conducted in the future (Ahmed et al., 2013; Baloch et al., 2024; Kumar et al., 2024). The present work completes this gap in our knowledge by examining 20 varying barley genotypes both in normal as well as drought environments keeping in mind the coordination of anatomical as well as physiological traits. The aims were to (i) analyze genotypic variation in significant drought-responsive anatomical as well as physiological traits, (ii) determine the effect of drought stress on these traits, and (iii) identify genotypes having enhanced adaptation responses that can be targeted further in breeding programs in the future.

MATERIAL AND METHODS

This study involved the assessment of 20 barley genotypes under both optimal and drought-induced stress environments, employing a Randomized Complete Block Design (RCBD) with three replications to ensure statistical robustness. The field experiment was carried out at the research farm of the Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur. Sowing was performed by dibbling two seeds per hill, followed by thinning to retain the most vigorous seedling post-emergence. Each genotype was cultivated in 2-meter-long rows, maintaining an intra-row spacing of 15 cm and inter-row spacing of 30 cm. This spatial arrangement facilitated optimal light interception and air circulation, thereby minimizing inter-plant competition. Under well-watered (non-stressed) conditions, irrigation was applied at key developmental stages - tillering (35 days after sowing, DAS), booting (85 DAS), and milking (112 DAS) - as per standard recommendations. Drought stress was introduced by withholding irrigation at the tillering stage, following the methodology described by (Noorka and Teixeira da Silva, 2014). Agronomic practices such as fertilization, hoeing, and weed control were uniformly implemented across both treatment conditions to minimize environmental variability and ensure the accuracy of genotype performance comparisons (Ahmed et al., 2018). Except for the imposed drought treatment, all other cultural operations were applied identically in both environments to maintain uniform growing conditions. Physiological data were recorded at crop maturity, selecting five representative plants per genotype in each treatment for trait evaluation, ensuring the samples captured the full spectrum of genotypic expression.

Measured Traits

The evaluation of physiological and anatomical traits was conducted to assess the performance of barley genotypes under both normal and drought-stressed conditions. Cell Membrane Thermostability (CMT) was measured using the electrolyte leakage method, employing the formulas proposed by (Blum and Ebercon, 1981) to estimate membrane damage under thermal stress. Total Chlorophyll Content (TCC), representing the photosynthetic capacity of the plants, was determined by recording SPAD values using a portable chlorophyll meter. For stomatal traits, the third fully expanded leaf from randomly selected plants was used to measure both stomatal frequency and size. Leaf strips were taken from the central region of the leaf and preserved in Carnoy’s solution (a mixture of 10% acetic acid, 30% chloroform, and 60% ethanol) to arrest physiological activity and bleach the tissue (Sheehan and Hrapchak, 1973). After 48 hours, the strips were peeled using a fine razor blade and observed under a 40× microscope to determine stomatal frequency. The same samples were then used to assess stomatal size under 10× magnification, where the length and width of three randomly selected stomata were measured using an ocular micrometer and adjusted using a calibration factor of 3.33 μm to calculate average size. Epidermal Cell Size (ECS) was also recorded from the same leaf strips at 40× magnification by measuring the length and width of epidermal cells, which provided insights into anatomical responses to drought. Additionally, Leaf Venation (LV) was quantified by counting the number of longitudinal veins visible in a fixed field of view under 10× magnification, offering an estimate of vascular development and potential adaptive mechanisms (Ahmed et al., 2022).

Statistical Analysis

The collected data for each trait were subjected to Analysis of Variance (ANOVA) to evaluate the significance of differences among genotypes and treatment effects.

Statistical analysis was conducted in accordance with the procedures outlined by (Steel and Torrie, 1981), enabling identification of statistically meaningful variation attributable to genotype, environment, and their interaction.

RESULTS AND DISCUSSION

Analysis of variance of six physiological traits - Cell Membrane Thermostability (CMT), Total Chlorophyll Content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf Venation (LV), and Epidermal Cell Size (ECS) - among 20 barley genotypes grown in normal and drought stress environments showed highly significant differences among genotypes, environments, and genotypic-environment interactions (Table 1). These genotypic impacts were strongly significant ($p < 0.01$) on all traits, reflecting strong genetic variation among genotypes subjected to testing. This confirms there are large-scale physiological responses, which can be used in breeding activities to improve drought resistance. Environmental impacts were significant ($p < 0.05$ or $p < 0.01$), especially in SS, SF, and ECS, evidence of the dramatic influence of drought stress on plant physiological functions. Strongly significant genotypes × environments interactions on all these traits ($p < 0.05$ or $p < 0.01$) reveal that genotypes performed differently in normal and drought environments, also reflecting the need to test genotypes in more than a single environment in order to make effective selection possible. The same was also found by (Hasanuzzaman et al., 2022), who reported significant G×E interaction in barley under water stress conditions. Likewise, (Makhtoum et al., 2022) have also seen significant genotypic variation and environmental influence over physiological traits in barley, reaffirming these findings here. The high F-values of SF as well as ECS also reveal physiological perturbation under drought stress, something which also agrees with (Moualeu-Ngangué et al., 2020), who have found that these traits were sensitive indicators of drought tolerance. The findings therefore affirm that both genetic constitution as well as environmental stress play significant roles in determining variation in physiological traits in barley, and that their interaction has to be a prime consideration while breeding climate-resilient cultivar.

Table 1. Analysis of variances for physiological traits in 20 barley genotypes under normal and drought conditions

Sources of Variation	DF	CMT	TCC	SS	SF	LV	ECS
Replications	2	12.37	9.54	11.48	7.26	9.12	8.33
Genotypes	19	27.14**	13.65**	15.89**	74.31**	73.21**	84.51**
Environments	1	19.82*	24.88*	841.57*	759.2*	145.2*	927.34*
Genotypes × Environments	19	15.47**	19.74**	16.23*	58.06**	60.13**	62.11**
Error	78	2.76	5.91	3.72	3.51	5.21	4.94
Total	119						

*Significant at 0.05 level = *, Highly Significant at 0.01 level = **.*

Descriptive statistics of physiological traits

Physiologic trait descriptive statistics under drought stress and normal treatments revealed intense variability across 20 barley genotypes, thereby reflecting genetic variation as well as environmental influences on adaptation responses (Table 2). Cell Membrane Thermostability was significantly lower in drought (mean: 54.70) than in normal treatment (mean: 59.85), exhibiting lower variability (CV = 1.16%). This reduction is indicative of membrane destabilization under osmotic stress, a phenomenon commonly reported in cereals during drought (Ahmed et al., 2013). However, the relatively low coefficient of variation in both environments suggests that CMT is a stable and reliable trait for selection. Similar patterns were noted by Farooq et al. (2017), who emphasized CMT as a key indicator of drought and heat tolerance.

Stomatal Size (SS) also exhibited a significant reduction from a mean of 3471.80

in normal to 3291.27 in drought, accompanied by reduced variability (CV = 0.55%) during drought. Smaller stomata during stress are highly likely a water-conserving adaptation to reduce water loss, consistent with physiological trade-offs reported by (Vaezi et al., 2010). However, larger stomata under normal growth can enable increased carbon assimilation and growth. Frequency of stomata (SF) was found to have a moderate variability (CV ≈ 2.8%) in both treatments but increased slightly in its mean in stress condition (14.40) than that in normal condition (14.01). Larger stomatal density in stress can enhance plasticity in gas exchange and is in agreement with adaptation strategy discussed by (Kiani-Pouya et al., 2020), who concluded that highly regulated stomatal fine-tuning has a key role in fluctuating vapor pressure deficits. Leaf Venation (LV) was slightly lower during drought (mean = 7.65) than during normal growth (mean = 7.87) but more unpredictable (CV = 2.54%) during stress. It may be decreasing since restricted vasculature development during water stress might be decreasing hydraulic conductivity. This observation concurs with (Ajalli and Salehi, 2012), who pointed to venation structure as playing a critical part in effective water conduction during stressful environments. ECS surprisingly increased during drought (mean = 2689.15) than normal (mean = 2591.80), perhaps to act as compensation to conserve more water or capture more light. Despite this increase, the trait was relatively stable across genotypes, indicated by a low CV (~2.3%). Such anatomical adaptation was consistent with a study by (Saad et al., 2014), where increased epidermal size was found to ensure abiotic stress physiological resistance. For most of these characteristics, relatively low coefficients of variation suggest that these physiological and anatomical characteristics are genetically controlled, and these characteristics can be employed confidently in selection. Consistency of performance on multiple characteristics lends weight to (Zare, 2012) opinion, as well as that of (El-Hashash and Agwa, 2018), that trait screening, in an integrative perspective, forms a significant approach for breeding drought-tolerant cultivars of barley. Our work was concurrent to (Hasanuzzaman et al., 2022) who suggested the utilization of multiple, supplementary physiological characteristics as indicators of superior genotypes in stress condition climates.

Table 2. Descriptive statistics of Physiological traits in 20 barley genotypes under normal and drought stress conditions

Parameters	Environments	CMT	TCC	SS	SF	LV	ECS
Minimum	Normal	58.54	58.34	3363.47	13.41	7.71	2489.33
	Drought	53.78	50	3259.47	13.61	7.21	2580.32
Maximum	Normal	61.54	61.34	3583.47	14.74	8.11	2689.33
	Drought	55.7	51.97	3321.47	15.07	7.94	2780.32
Mean	Normal	59.85	60.11	3471.80	14.01	7.87	2591.80
	Drought	54.70	50.90	3291.27	14.40	7.65	2689.15
Standard Deviation	Normal	0.94	1.00	63.45	0.40	0.13	60.17
	Drought	0.64	0.65	18.21	0.40	0.19	62.84
CV%	Normal	1.57	1.66	1.83	2.88	1.63	2.32
	Drought	1.16	1.29	0.55	2.77	2.54	2.34

Cell Membrane Thermostability (CMT), Total Chlorophyll content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf venation (LV), Epidermal cell size (ECS).

Comparative evaluation of studied barley genotypes

Relative evaluation of 20 barley genotypes using normal plus drought stress environments revealed similar physiological as well as anatomical tendencies, identifying genotypes with stable yield across environments (Table 3 and Table 4). Cell Membrane Thermostability (CMT), a critical marker of stress resistance, was significantly highest in G8 (60.62), G13 (60.56), and G17 (60.55) across both treatments, indicating higher membrane stability against heat and osmo-stress. Contrary to these, the lowest ever recorded values of CMT were exhibited by G7 (53.93), G11 (53.95), and G19 (53.99), indicating greater sensitivity to abiotic stress conditions. The results are also aligned with (Sallam et al., 2019), who identified greater values of CMT as evidence of greater cellular performance under abiotic stress. Likewise, Total Chlorophyll Content (TCC) also was well conserved in G8 (56.66), G17 (56.63), and G13 (56.43), indicative of high photosynthetic potential in addition to greater preservation of chlorophyll in drought stress, an indicator of high endurance in productivity.

Contrary to this, there was also presented by G7 (50.02), G11 (50.00), and G19 (50.02) strong breakdown of chlorophyll, in agreement with (Kumar et al., 2024), where breakdown of chlorophyll was reported as a drought symptom in cereal crops. Stomatal features also responded consistently, where SS was highest in G17 (3437.47), G8 (3449.47), and G13 (3437.97) to promote higher adaptation to increased gas exchange. Contrary to this, however, were recorded by G11 (3259.47), G7 (3260.47), and G19 (3264.47) lowest stomata, meaning lower uptake of CO_2 but more water conservation. These are similar to (Hunt et al., 2021) findings, where stomatal conductance was compromised at the expense of water-use efficiency. Stomata Frequency (SF) was also in a similar hierarchy, where more stomatal density in G13 (14.89), G17 (14.86), and G8 (14.83) would be favorable in response to flexible environments.

Less stomatal density was found in G11 (13.61), G19 (13.88), and G7 (13.91), being a mechanism of drought avoidance through a decrease in transpiration. It supports (Rahim et al., 2021) findings that more stomatal density enhances adaptability when coupled with effective regulation. In terms of structural traits, Leaf Venation (LV) was most pronounced in G8 (7.99), G13 (7.98), and G17 (7.95)**,** indicating efficient hydraulic conductivity. G7 (7.21), G11 (7.44), and G19 (7.45) lagged behind, potentially limiting nutrient and water transport under drought. The trend extended to Epidermal Cell Size (ECS)**,** where G8, G13, and G17 (all 2780.32) possessed the largest cell sizes, enhancing surface area for light capture and gas exchange. Meanwhile, G7 and G11 (2580.32), and G19 (2630.32) recorded the smallest ECS values, indicating limited adaptive capacity. These anatomical advantages are supported by (Lv et al., 2023), who emphasized their role in drought tolerance.

Overall, G8, G13, and G17 consistently outperformed others across all traits in both environments, making them ideal candidates for breeding programs targeting yield stability and stress resilience. Conversely, G7, G11, and G19 were the least performant and may be less suitable for cultivation in stress-prone regions. These results reinforce conclusions from (Lv et al., 2023; Abdelrady et al., 2024; Mohi-Ud-Din et al. 2024), who advocated for integrative trait-based screening to develop climate-resilient crop cultivars.

Table 3. Mean Values of 20 barley genotypes using Physiological traits under normal conditions

Genotypes	CMT	TCC	SS	SF	LV	ECS
G1	58.54	59.34	3403.47	13.68	7.71	2539.33
G2	58.87	59.34	3413.47	13.79	7.71	2589
G3	59.54	60.34	3453.47	14.12	7.81	2589.33
G4	59.54	60.34	3463.47	13.95	7.81	2589.33
G5	60.54	61.34	3523.47	14.29	8.01	2639.33
G6	59.67	60.34	3473.47	13.97	7.91	2589.33
G7	58.54	58.34	3393.47	13.57	7.71	2489.33
G8	60.54	61.34	3583.47	14.74	8.11	2689.33
G9	59.32	59.34	3423.47	13.68	7.71	2539.33
G10	59.54	59.34	3430.13	13.66	7.81	2539.33
G11	58.54	58.34	3363.47	13.41	7.71	2489.33
G12	59.54	59.34	3443.47	13.58	7.81	2539.33
G13	61.54	61.34	3563.47	14.59	8.01	2689.33
G14	60.54	61.34	3533.47	14.42	8.01	2639.33
G15	60.54	59.34	3483.47	13.85	7.91	2589.33
G16	60.54	60.34	3493.47	14.04	7.91	2589.33
G17	61.54	61.34	3553.47	14.68	8.01	2689.33
G18	60.54	61.34	3543.47	14.49	8.01	2639.33
G19	58.54	59.34	3383.47	13.49	7.71	2539
G20	60.54	60.68	3513.47	14.14	7.91	2639.33

Cell Membrane Thermostability (CMT), Total Chlorophyll content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf venation (LV), Epidermal cell size (ECS).

Table 4. Mean Values of 20 barley genotypes Physiological traits under drought conditions

Genotypes	CMT	TCC	SS	SF	LV	ECS
G1	54.01	50.47	3271.47	14.21	7.46	2656.99
G2	54.09	50.54	3279.47	14.22	7.49	2680.32
G3	54.38	51.17	3284.47	14.53	7.58	2680.32
G4	54.48	50.85	3291.47	14.45	7.65	2680.32
G5	55.26	51.45	3305.47	14.71	7.81	2730.32
G6	54.56	50.88	3291.47	14.48	7.7	2680.32
G7	53.92	50.02	3260.47	13.91	7.21	2580.32
G8	55.7	51.97	3315.47	14.91	7.89	2780.32
G9	54.1	50.4	3280.47	14.05	7.49	2630.32
G10	54.3	50.28	3281.47	14.01	7.52	2630.32
G11	53.9	50	3259.47	13.61	7.44	2580.32
G12	54.32	50.05	3282.47	13.95	7.57	2630.32
G13	55.57	51.69	3312.47	15.07	7.94	2780.32
G14	55.33	51.52	3305.47	14.72	7.85	2730.32
G15	54.82	50.61	3301.47	14.35	7.7	2680.32
G16	55.22	51.1	3302.47	14.52	7.71	2730.32
G17	55.56	51.92	3321.47	15.03	7.9	2780.32
G18	55.42	51.45	3309.47	14.55	7.89	2780.32
G19	53.78	50.02	3264.47	13.88	7.45	2630.32
G20	55.26	51.63	3304.47	14.77	7.81	2730.32

Cell Membrane Thermostability (CMT), Total Chlorophyll content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf venation (LV), Epidermal cell size (ECS).

Biplot analysis

The combined principal component analysis (PCA) biplots (Figures 1 and 2) offer critical insights into the multivariate relationships between physiological traits and the performance of 20 barley genotypes under both normal and drought stress conditions. Under normal conditions, PC1 and PC2 collectively explained 96.42% of the total variation, while under drought conditions, this cumulative variance slightly increased to 97.17%, indicating a robust dimensional reduction and effective trait discrimination across both environments. The clustering and directional alignment of genotypes with trait vectors provide a powerful visual and statistical tool to identify stable and superior performers. Notably, genotypes G8, G13, and G17 consistently aligned with the positive directions of major physiological traits such as Cell Membrane Thermostability (CMT), Total Chlorophyll Content (TCC), Stomata Size (SS), Stomata Frequency (SF), Leaf Venation (LV), and Epidermal Cell Size (ECS) in both environments. Their repeated proximity to these vectors reflects strong physiological integrity, efficient photosynthetic performance, and cellular-level resilience under water-deficit stress. For instance, higher CMT values are closely associated with the maintenance of membrane structure and function during heat or drought stress, minimizing lipid peroxidation and oxidative damage (Blum and Ebercon, 1981; Mohi-Ud-Din et al., 2024). Likewise, elevated TCC indicates robust chlorophyll biosynthesis, critical for sustained photosynthetic rates under stress, which has been previously linked to enhanced yield stability in cereals (Afshari-Behbahanizadeh et al., 2024).

Furthermore, traits like stomatal size and frequency play an essential role in regulating gas exchange and water use efficiency. Smaller stomata coupled with optimized frequency can limit water loss while maintaining adequate CO_2 uptake, providing an adaptive advantage under limited moisture availability (Boussora et al., 2024; Ayaz et al., 2025). Genotypes G8, G13, and G17 likely possess such adaptive stomatal traits, making them suitable for drought-prone environments. Increased vein density (LV) is another trait contributing to efficient hydraulic conductance and photosynthate transport, crucial for growth and recovery under stress, as supported by (Cheng et al., 2025). Conversely, genotypes G7, G11, and G19 consistently clustered in the negative quadrants of the biplots, showing poor association with most trait vectors. Their spatial separation from traits like CMT and TCC implies lower physiological adaptability and potential susceptibility to drought-induced stress. These genotypes likely exhibit impaired membrane stability and chlorophyll degradation, which could hinder overall plant performance under water-limited conditions (Kumar et al., 2024; Ferioun et al., 2025). The trait vectors in both biplots revealed meaningful inter-trait associations. In both conditions, positive correlations were observed between TCC, SF, and ECS, suggesting a synergistic relationship among photosynthetic potential, epidermal adaptations, and gas exchange efficiency.

Under drought, these associations became even more pronounced, indicating that these traits are co-regulated or co-selected under stress, aligning with the findings of (Kumar et al., 2024), who emphasized the integration of multiple physiological indices for drought tolerance screening. Interestingly, a subtle shift in trait dominance was observed across environments. While under normal conditions, traits like CMT, LV, and SS had relatively longer vectors with stronger discriminatory power along PC1, under drought conditions, TCC, SF, and ECS gained more explanatory strength. This shift highlights the plasticity and environmental responsiveness of certain traits, reinforcing the notion that drought tolerance is a complex, multi-genic trait influenced by dynamic physiological pathways (Kurowska et al., 2025).

The biplots also confirmed that PCA is a powerful method not only for genotype differentiation but also for simplifying complex trait relationships into actionable breeding insights. The consistent performance of G8, G13, and G17 under both environments underscores their genetic stability and physiological robustness, making them ideal parental lines for developing high-yielding, drought-resilient barley cultivars. Such findings are in line with studies by (Al-Ashkar, 2024; Rahmati et al., 2024) who advocate for PCA and biplot-based selection strategies in crop improvement programs. The integrated biplot analysis effectively elucidates the physiological trait contributions and genotype stability under contrasting water regimes (Rahim et al., 2021). It highlights specific genotypes and traits with the potential to serve as selection indices in breeding programs aimed at enhancing drought tolerance, yield stability, and climate resilience in barley. These results serve as a basis for further molecular and physiological investigations to validate and utilize these promising genotypes in targeted breeding pipelines.

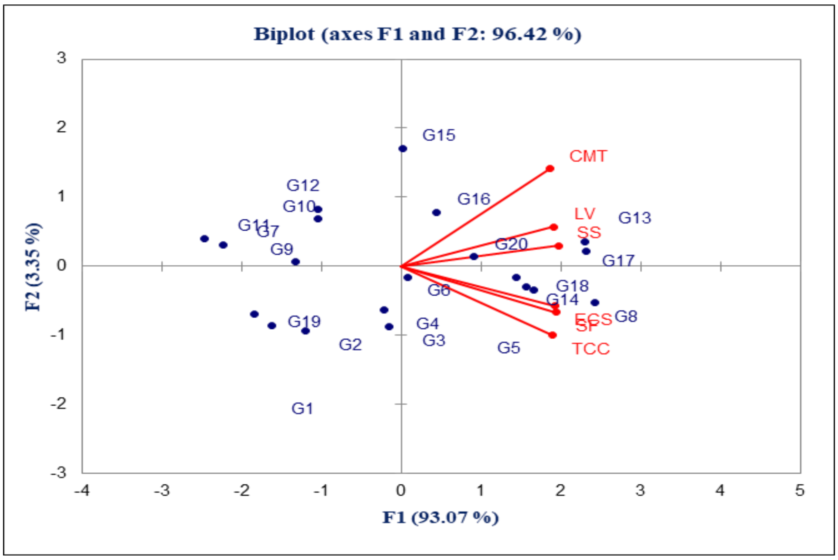

Figure 1. Biplot (Genotypes X Traits) of 20 barley genotypes for physiological traits under normal conditions

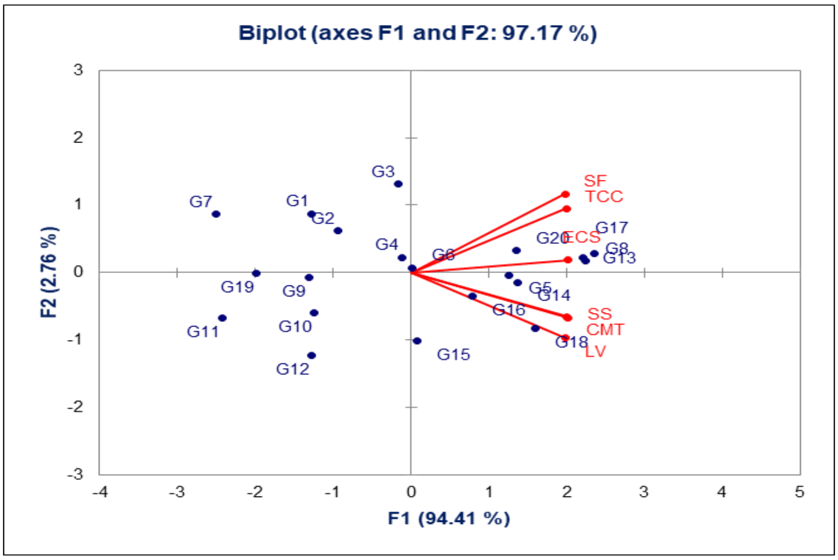

Figure 2. Biplot (Genotypes X Traits) of 20 barley genotypes for physiological traits under drought conditions

CONCLUSIONS

The integrative evaluation of 20 barley genotypes under contrasting moisture regimes revealed pronounced genetic variation and environment-induced physiological divergence, essential for breeding drought-resilient cultivars. All six physiological and anatomical traits - CMT, TCC, SS, SF, LV, and ECS - exhibited significant responses to drought, affirming their relevance as drought-responsive indicators. The substantial reduction in CMT and TCC under drought stress underscores compromised membrane stability and photosynthetic potential, while the increase in ECS likely reflects anatomical compensation for water deficit.

Stomatal size and frequency displayed adaptive modulation, with reduced SS and increased SF, promoting water conservation and stress responsiveness. Leaf venation declined marginally, suggesting restricted vascular development under stress. Biplot analysis delineated strong genotypic associations with physiological traits, with G8, G13, and G17 genotypes emerging as consistently superior across environments, supported by their elevated CMT, TCC, and ECS (2780.32), along with optimized stomatal and venation architecture. These genotypes are prime candidates for future breeding programs targeting drought tolerance. In contrast, G7, G11, and G19 genotypes exhibited suboptimal trait expression, rendering them less suitable for stress-prone agroecology. The study highlights the importance of high-resolution phenotyping and multivariate screening for enhancing crop resilience in water-limited environments. Future efforts should integrate these physiological insights with genomic and transcriptomic tools to accelerate the development of climate-smart barley cultivars capable of sustaining yield under abiotic stress.

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