Genetic variability and phenotypic associations of physiological and agronomic traits in bread wheat genotypes under drought-stressed and well-watered conditions (#112267)

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Genetic variability and phenotypic associations of physiological and agronomic traits in bread wheat genotypes under drought-stressed and well-watered conditions

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Drought is a critical abiotic stress significantly reducing global wheat production, in particular under climate fluctuations. Investigating wheat genetic variability using physiological and agronomic characteristics is essential for advancing breeding to enhance drought resilience and ensure sustainable production in light of global population growth. The genetic diversity and associations among traits of fourteen diverse genotypes of bread wheat in drought-stressed and well-watered conditions were studied focusing on physiological and agronomic responses. Significant variations were detected among irrigation regimes, genotypes, and their interactions for all assessed characteristics. Drought stress substantially declined chlorophyll *a*, and *b*, net photosynthetic rate, transpiration rate, stomatal conductance, membrane stability index, relative water content, plant height, yield-related attributes and grain yield. Conversely, it significantly increased malondialdehyde content, proline content, and activities of antioxidant enzymes, including catalase, ascorbate peroxidase and superoxide dismutase. The evaluated wheat genotypes exhibited significant variations in physiological, biochemical, and agronomic performance under well watered and drought conditions. The heatmap and cluster analyses distinctly separated the genotypes based on their performance under

water deficit conditions into different groups representing a gradient from drought sensitivity to tolerance. Genotypes G3, G8, and G12 exhibited the highest drought tolerance indices, identifying them as promising candidates for future breeding efforts to develop drought-resilient and high-yielding wheat genotypes. Principal component and correlation analyses identified chlorophyll content, relative water content, membrane stability index, photosynthetic efficiency, and antioxidant enzyme activities as critical traits associated with drought resilience and grain yield. The direct and indirect path analysis emphasized the importance of these characters as significant contributors to grain yield. Moreover, these traits demonstrated high heritability under drought stress, suggesting the feasibility of effective indirect selection in water-limited environments. Therefore, these characteristics could be identified as key selection indirect criteria for breeding drought-resilient wheat. This study depicted the potential of exploring genetic variability and key physiological traits to improve drought tolerance in bread wheat and ensure sustainable agricultural productivity under water-limited environments.

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Abstract

Drought is a critical abiotic stress significantly reducing global wheat production, in particular under climate fluctuations. Investigating wheat genetic variability using physiological and agronomic characteristics is essential for advancing breeding to enhance drought resilience and ensure sustainable production in light of global population growth. The genetic diversity and associations among traits of fourteen diverse genotypes of bread wheat ~~in~~ drought-stressed and well-watered conditions were studied focusing on physiological and agronomic responses. Significant variations were detected among irrigation regimes, genotypes, and their interactions for all assessed characteristics. Drought stress substantially declined chlorophyll *a*, and *b*, net photosynthetic rate, transpiration rate, stomatal conductance, membrane stability index, relative water content, plant height, yield-related attributes and grain yield. Conversely, it significantly increased malondialdehyde content, proline content, and activities of antioxidant enzymes, including catalase, ascorbate peroxidase and superoxide dismutase. The evaluated wheat genotypes exhibited significant variations in physiological, biochemical, and agronomic performance under well watered and drought conditions. The heatmap and cluster analyses distinctly separated the genotypes based on their performance under water deficit conditions into different groups representing a gradient from drought sensitivity to tolerance. Genotypes G3, G8, and G12 exhibited the highest drought tolerance indices, identifying them as promising candidates for future breeding efforts to develop drought-resilient and high-yielding wheat genotypes. Principal component and correlation analyses identified chlorophyll content, relative water content, membrane stability index, photosynthetic efficiency, and antioxidant enzyme activities as critical traits associated with drought resilience and grain yield. The direct and indirect path analysis emphasized the importance of these characters as significant contributors to grain yield. Moreover, these traits demonstrated high heritability under drought stress, suggesting the feasibility of effective indirect selection in water-limited environments. Therefore, these characteristics could be identified as key selection indirect criteria for breeding drought-resilient wheat. This study depicted the potential of exploring genetic variability and key physiological traits to improve drought tolerance in bread wheat and ensure sustainable agricultural productivity under water-limited environments.

Keywords: Climate resilience; genetic variability; wheat genotypes; selective breeding; drought tolerance; multivariate analyses; agronomic performance; physiological traits.

Introduction

Wheat (*Triticum aestivum* L) is one of the most commercially significant and widely cultivated crops in the world *(Mokhtari et al. 2024)*. It is cultivated on 220.4 million hectares, producing approximately 798.9 million tons annually *(FAOSTAT. 2023)*. It is a dietary staple in many countries, primarily used for bread, and various baked products *(Mesta-Corral et al. 2024)*. Furthermore, its straw is utilized for animal feed and in the manufacturing of diverse industrial products *(Kamara et al. 2021)*. Wheat grains are distinguished by their high carbohydrate content, which is a vital source of energy. ~~Wheat grains also provide dietary~~ fiber, fats, essential minerals and vitamins *(Moustafa et al. 2021)*. Its adaptability and nutritional advantages are fundamental components of global diets, contributing to food security and economic resilience. Wheat production should be improved to meet the dietary needs of growing global population *(Gahlaut et al. 2017; Neupane et al. 2022)*. Nevertheless, current yield improvements, especially in dry-land farming systems, are limited *(Lopes et al. 2012; Thungo et al. 2020)*.

Climate fluctuations pose a significant challenge to global wheat production *(Asseng et al. 2013; Rezaei et al. 2023)*. Increasing temperatures and frequent variations in precipitation are expected to increase drought severity worldwide *(Bracho-Mujica et al. 2024; Cramer et al. 2018; Hou et al. 2024)*. ~~The~~ water deficit is a severe environmental challenge that significantly impacts wheat production *(Leng & Hall 2019; Mao et al. 2023)*. It induces biochemical, physiological, and morphological alterations in the plants, disrupting their growth and development *(Farooq et al. 2024)*. The duration, intensity, and timing of ~~the~~ water deficit ~~relative to plant stage~~ determine the severity of the destructive effects of drought stress *(Wang et al. 2022)*. ~~Specifically,~~ cell dehydration caused by water scarcity restricts cell elongation, induces stomatal closure, reduces photosynthetic efficiency, and restricts overall plant growth and development *(Farooq et al. 2009; McAusland et al. 2020)*. The primary way that plants respond to water scarcity is through closing their stomata that reduces water loss ~~from plant~~ leaves *(Qiao et al. 2024)*. However, this adaptation limits CO_2 assimilation, reducing photosynthetic efficiency *(Li et al. 2017)*. Moreover, reactive

oxygen species (ROS) are produced by imbalanced photochemical reactions in chloroplasts, which result in surplus light energy that is not effectively used in photosynthesis. The oxidative stress damages cellular structures, including membranes, through lipid peroxidation *(Sachdev et al. 2021)*. This damage impairs growth, disrupts mineral uptake, and compromises photosynthetic activity. To reduce ROS production, the plants activate antioxidant defense systems. In order to deactivate ROS and preserve cellular integrity, enzymes involving superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are essential. SOD serves as the first line of defense by producing hydrogen peroxide ($H2O_2$) and oxygen (O_2) from superoxide radicals (O_2^-). Subsequently, CAT and APX detoxify H_2O_2, preventing oxidative damage *(Gill & Tuteja 2010)*. Additionally, drought stress often leads to increased accumulation of proline, a key osmoprotectant *(Zia et al. 2021)*. Proline stabilizes cell membranes, mitigates oxidative damage, and supports plant ability to tolerate water scarcity *(Shafi et al. 2019)*.

Developing high-yielding, drought-tolerant cultivars is a critical and effective strategy to alleviate the deleterious effects of water scarcity. This approach involves utilizing new genetic resources that exhibit favorable physiological and agronomic traits strongly correlated with grain yield. These traits are essential for enhancing selection efficiency, particularly in water-limited environments. Traditional wheat breeding methods often rely on univariate statistical approaches *(Gilmour et al. 1997)*. While applicable, these methods are limited in addressing the complex interactions among traits, stress factors, and the need to evaluate multiple attributes simultaneously for optimal selection. In contrast, advanced statistical models, particularly multivariate analyses like principal component analysis (PCA) and cluster analysis have significantly improved the reliability and efficiency of the selection process *(Galal et al. 2023)*. Understanding the relationships between grain yield and various physiological parameters can significantly enhance effectiveness of breeding programs. Breeders can develop more targeted and efficient screening methods by identifying key traits that are reliable selection tools under drought-stressed and normal conditions. Characteristics including proline accumulation, antioxidant activity, relative

water content, and chlorophyll concentration are beneficial as secondary markers for determining drought-tolerant genotypes *(Ahmed et al. 2020)*. Integrating these agronomic and physiological traits into breeding programs provides a comprehensive approach to addressing drought stress. This ensures that the resulting genotypes not only achieve high grain yields but also exhibit robust resilience to water scarcity *(Sallam et al. 2024)*. This work aimed to evaluate physiological and agronomic responses of diverse wheat genotypes under drought and well watered conditions. Additionally, the study aimed to identify wheat genotypes demonstrating consistent high-yield potential and strong drought tolerance and analyze the associations among the studied parameters in normal and water-deficit conditions.

MATERIALS &METHODS

Experimental site

Field experiment was conducted during two growing seasons of 2021-2022 and 2022-2023 at the Experimental Filed, Kafr El-Sheikh governorate, located at north Egypt (31° 6′N, 30° 56′E). The climate at the experimental location is arid, with an average of about 40 mm of rainfall per year. Figure S1 displays meteorological information for both growth seasons. The soil was characterized as clay (49% clay, 14.5% sand, and 36.5 silt) throughout the profile. In addition, the soil water parameters included a permanent wilting threshold of 20.3%, a field capacitance of 37.2%, and an accessible water content of 16.4% (Table S1).

Plant materials

Fourteen diverse bread wheat genotypes, selected for their variability in yield and drought tolerance, were evaluated in this study. The Egyptian Agricultural Research Center and the International Maize and Wheat Improvement Center (CIMMYT) provided these genotypes. The genotypes included six advanced breeding lines, five exotic genotypes from CIMMYT, and three high-yielding commercial cultivars featured on the Egyptian recommended list. Detailed information on the pedigrees and origins of these genotypes is provided in Table S2.

Experimental design and agronomic practices

Two different irrigation treatments were used to evaluate the studied wheat genotypes. The first treatment was well-watered conditions, which applied 4448 m³/ha of water over five irrigations during the growing season. With a total water application of 2865 m³/ha, the second one, which simulated drought stress conditions, only received two irrigations over the season. This study applied split-plot design in three replicates in a randomized block arrangement. The irrigation treatments were assigned to the main plots, while the wheat genotypes were allocated in the sub-plots. Six rows in each plot, each three meters long with 20-cm between rows. Before sowing, a single dose of 35 kg P ha^{-1} phosphorus fertilizer was applied. Three separate applications of nitrogen fertilizer were added at sowing, 30 days following sowing and the tillering stage, with a total amount of 180 kg N/ha.

Measured traits

Chlorophyll content and photosynthetic efficiency

Chlorophyll *a* and *b* were measured by centrifuging 0.5 g of leaf samples homogenized in 5 mL of 85% cold acetone. Following *Lichtenthaler (1987)*, the optical density was measured using spectrophotometry at 663 and 647 nm after the resulting extract were diluted to the appropriate volume. A portable steady-state parameter (LI-1600, LICOR, Lincoln, NE, USA) was utilized to determine photosynthetic parameters; net photosynthesis rate (NPR), transpiration rate (Tr), and stomata conductance (gs). To ensure the precision ~~of~~ gs was recorded on a fully inflated flag leaf using three replicates per leaf. The formula A=Amax×f(PAR), where Amax is the maximum theoretical photosynthetic rate and f(PAR) is a function of photosynthetically active radiation (PAR) recorded using a calibrated quantum sensor at three different times (morning, noon, and afternoon), was utilized to calculate NPR *(Sicher & Barnaby 2012)*. The same leaves were used to measure Tr, considering both the adaxial and abaxial surfaces directly.

Water relations and malondialdehyde

Relative Content of Water (RWC) was determined using the method of *Barrs & Weatherley (1962)*. The youngest leaf tissues (0.2 g), were cut into 1 cm pieces after cleaning with deionized water, and submerged in 10 mL of deionized water, which were utilized to calculate membrane stability index (MSI). After that, these samples were cooked for 30 minutes at 40 °C in a water bath. After that, a conductivity meter (ME977-C, Max Electronics) was used to re-order the electrical conductivity (EC1). The electrical conductivity (EC2) of the samples was then determined after they had been cooked for ten minutes in a water bath at 100 °C *(Premachandra et al. 1990)*. A spectrophotometer measured lipid peroxidation as malondialdehyde (MDA) at µmol/g FW *(Davenport et al. 2003)*. An equivalent volume of a solution comprising 0.5% (w/v) thiobarbituric acid and 5% (w/v) trichloroacetic acid was mixed with the leaf extract. After 15 minutes of heating at 100°C, the mixture was centrifuged for 10 minutes at 12,000 rpm. A spectrophotometer was then used to measure the absorbance of the resultant supernatant at wavelengths of 532, 600, and 450 nm.

Proline and enzymatic antioxidants activities

Proline content was calculated utilizing the procedure outlined in *Bates et al. (1973)*. The procedure involved extracting 0.5 g of plant tissue in 5% sulfosalicylic acid and centrifuging it for 7 minutes at 10,000×g. The resultant supernatant was boiled for 30 minutes at 94°C after being diluted with water and combined with 2% ninhydrin reagent. Toluene was added to the mixture after it had cooled, and the upper organic phase was examined using spectrophotometry at 520 nm. To determine antioxidant enzyme activity, 200 mg of leaf samples were quickly frozen in liquid nitrogen and then crushed in 2.0 mL of an extraction buffer that contained 10 mM ascorbic acid, 100 mM potassium phosphate (pH 7.8) and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The final homogenate was centrifuged for 15 minutes at 4 °C at 13,000 × g. Protein content and enzyme activity were then measured using the supernatant left over after centrifugation. According to *Aebi (1984)* CAT activity was measured in the supernatant at 240 nm based on H_2O_2 consumption. Monitoring the drop in absorbance at 290 nm allowed for measuring APX activity following *Ma*

190 *& Cheng (2004)*. Using the approach of *Giannopolitis & Ries (1977)*, the SOD activity was
191 determined at 560 nm.

192 **Agronomic traits**

193 The vertical distance from soil to spike tip (omitting awns) was used to calculate the plant
194 height (cm). Ten spikes were chosen randomly from each plot to determine number of grains/spike.
195 A sample of 1000 grains was weighed to determine the thousand-grain weight (g). All harvested
196 plants from each plot were threshed, the grains were weighed, and the measurement was converted
197 to tons of ha^{-1} to determine the grain yield (ton ha^{-1}).

198 ***Statistical analysis***

199 The tests of Shapiro-Wilk and Bartlett were used to check the homogeneity of variances and
200 the normality of residuals before the ANOVA was performed *(Bartlett 1937; Shapiro & Wilk*
201 *1965)*. To assess differences between the irrigation regime, genotype, and their interaction, the
202 least significant difference (LSD) test ($p < 0.05$ and < 0.01) was used. RStudio 4.1.1 was used to
203 create the figures. The FactoExtra package was employed to perform Principal Component
204 Analysis (PCA), and the Com-plexHeatmap package was utilized to create a heatmap. Using the
205 procedures outlined by *Burton & De Vane (1953)*, the genotypic and phenotypic variance
206 components and their coefficients of variation were calculated. The methods given by *Allard*
207 *(1999)* were used to estimate genetic advancement and broad-sense heritability. Drought tolerance
208 indices (Table S3) were computed to classify the genotypes according to their drought tolerance,
209 also cluster analysis was carried out using these indicators.
210

Results

Analysis of variance

Combined variance analysis revealed that genotypes and irrigation exhibited highly significant effects on all physiological and agronomic characteristics, highlighting the substantial role of genetic makeup and environmental factors on these traits (Table 1). Significant interactions between genotypes and irrigation were detected across all evaluated characters, underscoring the necessity of studying genotype-by-environment interactions when developing strategies for crop improvement. The three-way interaction between genotypes, irrigation, and growing seasons was insignificant for grain yield and most assessed characters.

Physiological and agronomic performance

Sixteen physiological and agronomic characters were studied under normal and drought-stressed conditions. Significant variations were observed across all traits between irrigation treatments. Under drought conditions, a substantial reduction was observed in Chl*a*, Chl*b*, Tr, NPR, gs, RWC, MSI, PH, NGPS, and GY (Figure 1). In contrast, traits such as MDA, Proc, CAT, APX, and SOD showed significant increases under water deficit conditions, suggesting their role in drought tolerance mechanisms. The genotypes exhibited substantial differences in all characters under drought and well-watered conditions, emphasizing genetic variability and its potential for breeding programs.

Physio-biochemical attributes

The physio-biochemical attributes of the evaluated fourteen wheat genotypes are illustrated in Figures 2-4. Under normal conditions, chlorophyll *a* (Chl*a*) ranged from 3.48 to 3.77 mg/g FW, with genotype G3 showing the highest value, followed by G8, G4 and G11 (Figure 2A). While under drought conditions, Chl*a* values decreased significantly, ranging from 2.36 to 2.81 mg/g FW, with G10, G11, G12 and G8 performing the best. In normal conditions, Chl*b* ranged between

1.49 and 2.65 mg/g FW, with G11, G12, G3 and G9 demonstrating the highest value (Figure 2B). Under drought stress, Chl*b* values were reduced to 1.32 to 1.88 mg/g FW, with G12, G13, G11, G8 and G3 performing best. Net photosynthetic rate (NPR) ranged from 16.48 to 25.75 g/m² under normal conditions, with genotypes G3, G9, G1 and G4 recording the highest rate (Figure 2C). Under drought stress, NPR declined to 12.02 to 17.44 g/m², where genotypes G1, G8, G6 and G9 exhibited the best performance. Transpiration rate (Tr) ranged from 3.57 to 4.56 µmol CO_2/m²/s in normal conditions, with G6, G5, G2, and G1 recording the highest value, and decreased to 2.17 to 4.04 µmol CO_2/m²/s under drought, where G2, G14, G5, and G7 exhibited the highest rate (Figure 2D). Stomatal conductance (gs) fluctuated from 0.48 to 0.90 cm²/S in normal conditions, with G8, G11, G12, G1, and G3 performing best, and reduced to 0.34 to 0.66 cm²/S under drought, where G3, G11, G6, and G8 excelled (Figure 2E). Relative water content (RWC) varied between 69.94% and 86.35% in normal conditions, with G3, G6, G5, and G11 achieving the highest value and declined to 54.76% to 64.54% under drought, with G8, G3, G12, and G13 showing superior performance (Figure 2F). Membrane stability index (MSI) ranged from 46.50% to 60.94% in normal conditions, with G1, G8, G9, and G3 excelling, and dropped to 32.83% to 51.96% under drought, where G3, G4, G12, and G8 demonstrated the best stability (Figure 3A). Malondialdehyde (MDA) content ranged from 23.19 to 46.89 µmol/g FW in normal conditions, with G6, G5 and G4 showing the highest, while G3, G10, and G13 had the lowest content (Figure 3B). Under drought, MDA increased significantly to 49.93 to 60.09 µmol/g FW, with G5, G14, and G13 recording the highest while G1, G8 and G9 recorded the lowest values. Proline content (Proc) ranged from 0.58 to 0.73 µmol/g DW in normal conditions, with G3, G7, G11, and G6 performing best, and increased to 1.54 to 2.15 µmol/g DW under drought, with G8, G3, G10, G13, and G12 exhibiting the highest content (Figure 3C). Catalase activity (CAT) ranged from 7.63 to 12.03 units/mg protein in normal conditions, with G6, G13, G7, and G8 performing best, and increased to 10.79 to 18.69 units/mg protein under drought, where G5, G6, G8, and G12 showed the highest activity (Figure 3D). Ascorbate peroxidase (APX) activity ranged from 4.88 to 9.62

263 units/mg protein in normal conditions, with G6, G14, G5, and G3 recording the highest activity
264 and increased to 7.73 to 18.72 units/mg protein under drought, where G8, G12, G6, and G2
265 excelled (Figure 3E). Superoxide dismutase (SOD) activity ranged from 33.29 to 40.20 units/mg
266 protein in normal conditions, with G14, G3, G10, G1, and G8 performing best, and increased to
267 53.90 to 68.59 units mg^{-1} protein under drought, where G8, G10, G3, and G2 demonstrated
268 superior activity (Figure 3F).
269

270 ***Agronomic traits***

271 Plant height (PH) ranged from 79.21 to 108.17 cm in normal conditions, with G3, G8, G4 and
272 G6 produced the tallest plants and decreased to 62.29 to 90.02 cm under drought, where G3, G4,
273 G8 and G1 performed best, while G14, G13, and G11 had the shortest plants (Figure 4A). Number
274 of grains/spike (NGPS) fluctuated from 53.86 to 74.72 in normal conditions, with G4, G3, G11,
275 and G8 achieving the highest value, and dropped to 40.74 to 61.02 under drought, with G3, G8,
276 G6, and G10 showing the best performance (Figure 4B). Thousand kernel weight (TKW) varied
277 from 40.92 to 54.55 g in normal conditions, with G4, G8, G6, G5, and G12 producing the heaviest
278 kernels, and decreased to 31.89 to 41.67 g under drought, where G8, G3, and G1 exhibited
279 superiority (Figure 4C). Grain yield (GY) ranged from 5.14 to 8.22 tons/ha in normal conditions,
280 with G3, G4, G8, and G1 achieving the highest yield and reduced to 3.51 to 6.32 tons/ha under
281 drought, where G8, G12, G3, and G6 outperformed other genotypes (Figure 4D).
282

283 ***Genotypic classification***

284 The heatmap analysis displayed distinct patterns in the performance of 14 bread wheat
285 genotypes (G1–G14) based on physio-biochemical attributes and agronomic characters under
286 water deficit conditions (Figure 5). The genotypes are clustered into three groups. Cluster 1 (G5,
287 G7, G2, and G14) demonstrated high levels of transpiration rate and malondialdehyde content.
288 Cluster 2 (G3, G8, G12) is characterized by high chlorophyll content and photosynthetic rate
289 indicating strong photosynthetic efficiency, also high proline levels and antioxidant enzyme

activities (APX, CAT, SOD), making these genotypes highly resilient to drought conditions. Besides, this cluster displayed high grain yield and its contributing traits. Cluster 3 (G1, G4, G6, G9, G13, G10, G11) showed moderate levels across most traits. These groupings highlight the genotypes varying capacities to balance stress resilience and yield performance. Genotypes such as G3, G8 and G12 are ideal candidates for breeding drought-tolerant wheat due to their superior physiological, biochemical and agronomic responses, while G2, G5, G7, and G14 require favorable conditions to exhibit their yield potential. Grouping the assessed traits emphasized their importance in genotype differentiation. Chlorophyll content (Chl *a* and Chl *b*), antioxidant enzymes (CAT, APX, SOD), and RWC were associated under drought stress. The attributes of photosynthetic parameters, water-retention traits and antioxidant enzymes are critical for maintaining growth under environmental stresses. Yield traits (GY, NGPS, TKW) were grouped together and were associated with certain physiological traits as RWC, MSI, NPR, SOD and Proc.

Drought tolerance indices

The assessed genotype was further classified using the computed drought tolerance indices, stress tolerance index, geometric mean productivity, mean productivity, harmonic mean, and yield index (Table S4). The hierarchical clustering analysis categorized the genotypes into four groups (Figure 6). Group A contained three genotypes (G8, G3 and G12) that exhibited the highest tolerance indices, classifying them as drought-tolerance genotypes. These genotypes exhibited strong adaptability to drought conditions and represent valuable candidates for wheat breeding to enhance drought tolerance. Group B comprised four genotypes (G6, G4, G1, and G11) demonstrating moderate tolerance index values. These genotypes displayed intermediate performance under drought stress. In contrast, five genotypes (G13, G10, G9, G5, and G7) in Group C, followed by two genotypes (G2 and G14) in Group D that displayed the lowest tolerance index, and were identified as drought-sensitive genotypes.

Ranking and AMMI analyses

317 The ranking biplot provides a detailed evaluation of stability and performance of 14 wheat
318 genotypes (G1–G14) across four distinct environments (E1–E4) under well-watered and water
319 deficit conditions across two seasons (Figure 7A). The first principal component (PC1) captured
320 74.07%, and the second principal component (PC2) explained 19.58% of the total variation of
321 genotype by environment interaction. The Average Environment Coordinate (AEC, represented
322 by a blue arrow) assesses the performance and stability of genotypes. Genotypes closer to the AEC
323 and aligned along its axis exhibited high performance and stability, while those far from the axis
324 were either less stable or showed environment-specific responses. Genotypes exhibited varying
325 levels of performance and stability across the environments. Genotypes G6, G3, G8 and G12 are
326 positioned close to the AEC axis, indicating high mean performance and stability across all
327 environments. These broadly adaptable genotypes can be suggested for cultivation under normal
328 and water deficit conditions. In contrast, genotypes such as G2, G14, and G13 located far from
329 AEC are better suited for regions with favorable growing conditions. The environments showed
330 variability in their alignment with the AEC, reflecting their distinctiveness and representativeness
331 of the overall mean performance. Environments E1 and E3, corresponding to normal conditions in
332 the first and second seasons, respectively, are located on the positive side of PC1. These
333 environments are favorable for wheat production as they align with high-yielding genotypes. In
334 contrast, E2 and E4, representing drought conditions in the first and second seasons, are situated
335 on PC1 negative side.

336 Also, in AMMI analysis, the four environments (E1-E4) showed distinct interaction patterns
337 with the genotypes (Figure 7B). E1 is located in the sector of positive PC1 and PC2, indicating
338 favorable growing conditions that associated with G1 and G2, which performed well under these
339 conditions. In contrast, E2 represents drought conditions in the first season in the negative PC2
340 sector. Genotypes G13 and G8 were associated with E2, suggesting their resilience to drought
341 stress in the first season. E3 corresponds to normal conditions in the second season, is placed in
342 the sector of positive PC1 and negative PC2, and is associated with G3. E4 represents drought

conditions in the second season and is situated in the sector of negative PC1 and PC2, with G12 strongly associated highlighting its performance under drought conditions. The genotypes displayed different levels of stability and adaptability across the studied environments. Genotypes G5, G6, G9, and G7 are located close to the biplot origin indicating minimal interaction effects and broad adaptability. These genotypes were stable across normal and drought conditions, which indicated their suitability for diverse environmental conditions.

Association analyses

Principal component analysis (PCA) was applied to explore the relationships among studied physiological and agronomic characters under different conditions. The biplot (Figure 8A) segregated the normal and drought conditions indicating diverse associations. The first two PCs (PC1 and PC2) explained 79.94% of the total variance, with PC1 accounting for 69.42% and PC2 for 10.52% (Figure 8B). Traits such as APX, MDA, SOD, Proc, and CAT were associated with drought stress were presented in distinct blue cluster. In contrast, traits related to normal conditions were presented in the separated yellow cluster, indicating to their differing responses to environmental conditions. Regarding trait contributions, Chl *a* had the highest contribution to PC1 (8.23%), followed by RWC (8.0%) and Proc (7.56%). For PC2, Tr was the dominant contributor (22.5%), followed by GY (11.32%) (Figures 8B, C).

Spearman correlation matrix for physiological and agronomic parameters under well-watered and drought conditions is presented in Figure 9A and 9. The correlation analysis revealed different patterns under normal and drought conditions. Under normal conditions (Figure 9A), positive associations were detected between grain yield and several key characters, including Chl *a*, MSI, NPR, PH, NGPS, and TKW. These associations emphasized the significance of these characters in determining yield under normal conditions. Besides, Chl *a* exhibited a strong positive association with NPR, PH, and NGPS, indicating its role in influencing agronomic performance. Other positive associations included NPR with SOD and RWC with APX, suggesting that improved photosynthesis and water retention are associated with enhanced antioxidant activity. Conversely, Tr displayed a strong negative association with NPR and MDA, indicating that oxidative stress negatively impacts photosynthetic efficiency.

Under drought conditions (Figure 9B), GY exhibited significant positive associations with Chl *a*, Chl *b*, NPR, MSI, RWC, ProC, SOD, NGPS, and TKW. Also, TKW was positively associated with RWC, ProC, and APX. In addition, NGPS showed strong positive correlations with PH, RWC, and SOD. Other pairwise associations were identified, such as Chl *a* with Chl *b*, CAT with APX, ProC with SOD, and RWC with CAT. Negative correlations were also observed between GY and MDA.

Path analysis

The direct and indirect effects of studied physiological and agronomic characters on grain yield under water deficit conditions are presented in Table S5. Most traits positively affected grain yield, except MDA, which showed a negative direct effect. RWC, MSI, chlorophyll content (Chl *a* and Chl *b*) and antioxidant enzyme activities CAT, APX, and SOD demonstrated positive direct effects on GY. In addition to direct contributions, some traits influenced grain yield indirectly. For example, RWC enhanced grain yield indirectly by positively affecting traits such as photosynthetic efficiency and antioxidant enzyme activity. Similarly, antioxidant enzymes (CAT, APX, and SOD) indirectly affected grain yield by improving RWC and MSI. Agronomic characters, including TKW and PH, indirectly contributed to grain yield by associating with physiological traits such as MSI and chlorophyll content.

Heritability, variance component, and genetic advance

The phenotypic variance exceeded the genotypic variance in all studied traits under normal conditions (Table 2). Among the characteristics, PH and NGPS had the greatest values of GCV and PCV, while the lowest values were observed for Chl*a*, Proc, and gs. High PCV values were recorded for NGPS, PH, MSI, and TKW. In contrast, GY, Tr, and Proc exhibited moderate PCV values. Likewise, GCV was highest for APX, NPR, and MSI, while PH, TKW, Tr, and gs displayed moderate GCV values. Broad sense heritability estimates varied from moderate to high across the studied characters. GY and Proc exhibited moderate genetic advance and heritability. In contrast, NPR, SOD, APX, and PH displayed high heritability with genetic advance.

Similarly, phenotypic variance exceeded genotypic variance for all characters under drought stress. PCV and GCV values were uppermost for APX, MDA, and NPR, while the lowest values were observed for Chl *a*, gs, and GY. NGPS, PH, MSI, and TKW exhibited high PCV values, whereas moderate PCV values were recorded for Tr, gs, and Proc. Likewise, GCV was highest for APX, NPR, and MDA, while moderate GCV values were observed for PH, TKW, Tr, and GY. Broad sense heritability varied from moderate to high for studied characters. APX, SOD, MDA, and NGPS exhibited high heritability and genetic advance under drought stress. In contrast, GY, gs, and Proc showed moderate heritability and genetic advance.

Discussion

Climate change increases drought stress in arid Mediterranean regions and severely diminishes wheat yields *(Melki et al. 2024)*. Therefore, developing drought-tolerant genotypes is essential for sustaining wheat production. Screening genotypes for tolerance to water scarcity using physiological and agronomic traits under natural field conditions in targeted environments is an effective strategy for enhancing wheat breeding in arid environments. This study focused on bread wheat genotypes' physiological and agronomic performance under stressed and non stressed conditions in an arid environment. The ~~ANOVA~~ results demonstrated that both genotype and irrigation had a significant impact on all studied traits. These results displayed that the assessed materials possessed high degree of genetic variability, which could be employed in improving wheat productivity. These results are in consonance with *Morsy et al. (2022); Saidi et al. (2024); Tefera et al. (2021)*, who reported genetic variability among genotypes for physiological and agronomic characters under stressed and normal conditions. The observed significant genotype × irrigation interaction across studied traits highlighted the varied responses of genotypes to irrigation treatments. This result indicated the importance of studying these interactions in wheat breeding to develop resilient genotypes to diverse environmental conditions. Similarly, *Ru et al.*

(2024); Thapa et al. (2018); Upadhyay et al. (2023) depicted the vital effects of genotype-environment interactions on the expression of physiological and agronomic characters.

The results showed that water scarcity significantly reduced all assessed characters compared to normal conditions, except for antioxidant enzyme activities, proline content and malondialdehyde, which increased significantly. Drought stress significantly impacts the physio-biochemical pathways leading to impaired photosynthesis, reduced growth, and, ultimately, lower yields. The significant reduction in studied traits such as chlorophyll content (Chl*a*, Chl*b*), NPR, RWC, and MSI indicated that drought stress negatively affected photosynthetic capacity and water retention ability *(Kamara et al. 2022)*. Under drought conditions, the decrease of Chl*a* and Chl*b* under water deficit conditions indicates to of impair of the photosynthetic system *(Croce et al. 2024; Wang et al. 2017)*. Similarly, the reduction in NPR and Tr limited carbon dioxide assimilation and disrupted stomatal conductance, consequently decreased plant productivity *(Osakabe et al. 2014; Sallam et al. 2019)*. Moreover, increased levels of MDA indicated oxidative damage and disturbed cellular activities. Compared to the plants under normal conditions, elevated antioxidant activities and proline accumulation in stressed wheat plants may result from the direct action of free radical scavengers, emphasizing their vital function as drought stress tolerance mechanisms. By promoting osmotic adjustment, preserving redox equilibrium, and stabilizing proteins, proline, an essential osmolyte, protections plant cells *(Mwadzingeni et al. 2016)*. SOD is primary defense against oxidative damage which transforms O_2- radicals into H_2O_2 and O_2 *(Chung 2017)*. By breaking down H_2O_2 into water and oxygen, APX assistances with detoxification *(Sachdev et al. 2021)*, while CAT is essential for signal transduction and metabolism *(Zhang et al. 2022)*. Grain yield of the assessed genotypes ~~was~~ significantly reduced under drought stress compared to well-watered conditions. This reduction could be attributed to reduced assimilate availability, pollen abortion, impaired fertilization, and disrupted grain filling. This negative effect of water deficit on yield traits were documented in studies such as those by *Rijal et al. (2024)*,

449 *Khan et al. (2024)*, *Mutanda et al. (2024)*, *Wang et al. (2024)*, *Xu et al. (2023)* which emphasized
450 the sensitivity of yield components to water deficit.

451 The heatmap and cluster analyses displayed different responses of assessed genotypes to
452 water deficit. The genotypes were classified into different groups ranging from drought sensitive
453 to tolerant. Genotypes G3, G8, and G12 demonstrated superior performance under drought
454 conditions, characterized by higher chlorophyll content (Chl *a* and *b*), RWC, and enhanced
455 antioxidant enzyme activities (CAT, APX and SOD). These parameters displayed the ability of
456 these genotypes to sustain photosynthetic efficiency and cellular stability, contributing to higher
457 grain yields under drought stress. Moreover, elevated proline and antioxidant levels emphasized
458 their superior drought tolerance, because these characters are crucial in osmotic adjustment and
459 scavenging ROS. The strong performance under drought stress identifies these genotypes as
460 promising candidates for wheat breeding to enhance its productivity under limited water conditions
461 *(Ahmad et al. 2022)*. Furthermore, ranking and AMMI biplots displayed valuable information on
462 the stability and adaptability of these genotypes. Therefore, these multivariate analyses are
463 important in differentiating wheat genotypes based on physiological and agronomic traits under
464 stress conditions *(Al-Ashkar et al. 2021; Kamara et al. 2022; Khan et al. 2023; Upadhyay et al.*
465 *2023)*.

466 Direct or indirect selection of agronomic and physiological character can improve grain yield.
467 In this study, correlation analyses revealed strong positive associations between grain yield and
468 Chl a, MSI, NPR, PH, and NGPS under normal conditions. These associations indicated the
469 importance of photosynthetic parameters and structural traits in maximizing yield potential under
470 normal conditions. Otherwise, under water deficit conditions, grain yield displayed positive
471 associations with RWC, Proc, and antioxidant enzyme activities (CAT and SOD). This indicated
472 the vital role of physiological adaptations in mitigating the damages induced by water scarcity.
473 Consequently, grain yield could be improved by enhancing these physiological characteristics
474 under drought conditions. In this regard, the importance of physiological parameters as markers of

grain yield under water deficit was clarified by *Yasir et al. (2019)*, *Ahmad et al. (2018)*, and *Shirvani et al. (2023)*. Additionally, the negative correlation between grain yield and MDA observed under water deficit presented detrimental effects of lipid peroxidation on yield. This finding indicated the importance of selecting genotypes with lower MDA levels to enhance grain yield and drought tolerance *(Devi et al. 2024)*. The different correlations under normal and drought conditions revealed different strategic adjustments in resource allocation and physiological processes. Under normal conditions, traits were associated with growth and productivity, while under drought stress, traits such as antioxidant activity were related to stress resilience as depicted by *Foulkes et al. (2007); Pantha et al. (2024)*.

Assessing genetic variability and heritability of physiological and agronomic parameters under normal and water scarcity conditions is pivotal for developing drought tolerant wheat genotypes. In the present study, higher PCV compared to GCV for most characters under normal and water deficit conditions indicated significant effect of irrigation treatments on studied traits. These results align with the research of *Pour-Aboughadareh et al. (2020)*, *Mansour et al. (2023); Sewore & Abe (2024)*, which also stated significant variability and potential for selection across physiological and agronomic characters under both conditions. Heritability values were slightly higher under drought conditions than under normal conditions for most characters, suggesting that selection for moisture response is more feasible in stress environments *(Beyene et al. 2015)*. When combined with substantial genetic advances, high heritability suggests the presence of additive gene effects, indicating that selection could lead to meaningful genetic gains *(Johnson et al. 1955)*. Chl *b*, NPR, ProC, CAT, APX, SOD, and PH exhibited high genetic advance and moderately high heritability under drought conditions. This indicates their strong genetic potential for enhancing drought tolerance. Similarly *Ahmed et al. (2024); Fufa et al. (2024); Saeed et al. (2024)*, elucidated the critical role of exploring heritability and genetic advance of grain yield and related traits in enhancing selection strategies in wheat breeding under stress conditions.

Conclusions

The results displayed adverse effects of water deficit conditions on wheat productivity, with significant reductions in photosynthetic parameters, water retention ability, and yield traits. However, activating defense mechanisms, such as increased proline and antioxidant activities, displayed an important role in mitigating stress impacts. Genotypes G3, G8, and G12 exhibited superior resilience and consistent yield traits under drought stress. Therefore, these genotypes could be considered promising for improving drought resilience and ensuring sustainable wheat production in drought-prone regions. In contrast, the remaining moderately tolerant and sensitive genotypes require targeted improvement. Key traits, including chlorophyll content (Chl *a* and *b*), relative water content (RWC), photosynthetic efficiency (NPR, Tr and gs), and antioxidant enzyme activities (CAT, APX, SOD), were identified as crucial indicators of drought tolerance while reducing malondialdehyde (MDA) levels was essential for improving drought tolerance. These traits exhibited high heritability and genetic advance, providing a strong foundation for genetic improvement. Consequently, integrating these biochemical and physiological parameters with agronomic traits in wheat breeding programs could offer an efficient approach to improve drought tolerance and address the challenges of climate variability.

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Figure 1

Comparative boxplots of 16 physiological and agronomic measured traits under normal and drought conditions.

Comparative boxplots of 16 physiological and agronomic measured traits under normal and drought conditions. Chlorophyll a (mg/g FW), Chlb: Chlorophyll b (mg/g FW), NPR: Net photosynthetic rate (µmol CO2/m2/s, Tr: Transpiration rate (µmol CO2/m2/s), gs: Stomatal conductance (µmol CO2/m2/s), RWC: Relative water content (%), MSI: Membrane stability index (%), MDA: Malondialdehyde (µmol/g FW), Proc: Proline content (µmol/g DW), SOD: Superoxide dismutase (unit mg/ protein), CAT: Catalase (unit mg/ protein), APX: Ascorbate peroxidase (unit mg/ protein), PH: Plant height (cm), NGS: Number of grains /spike, TGW: 1000-grain weight (g), and GY: Grain yield (tons/ha).

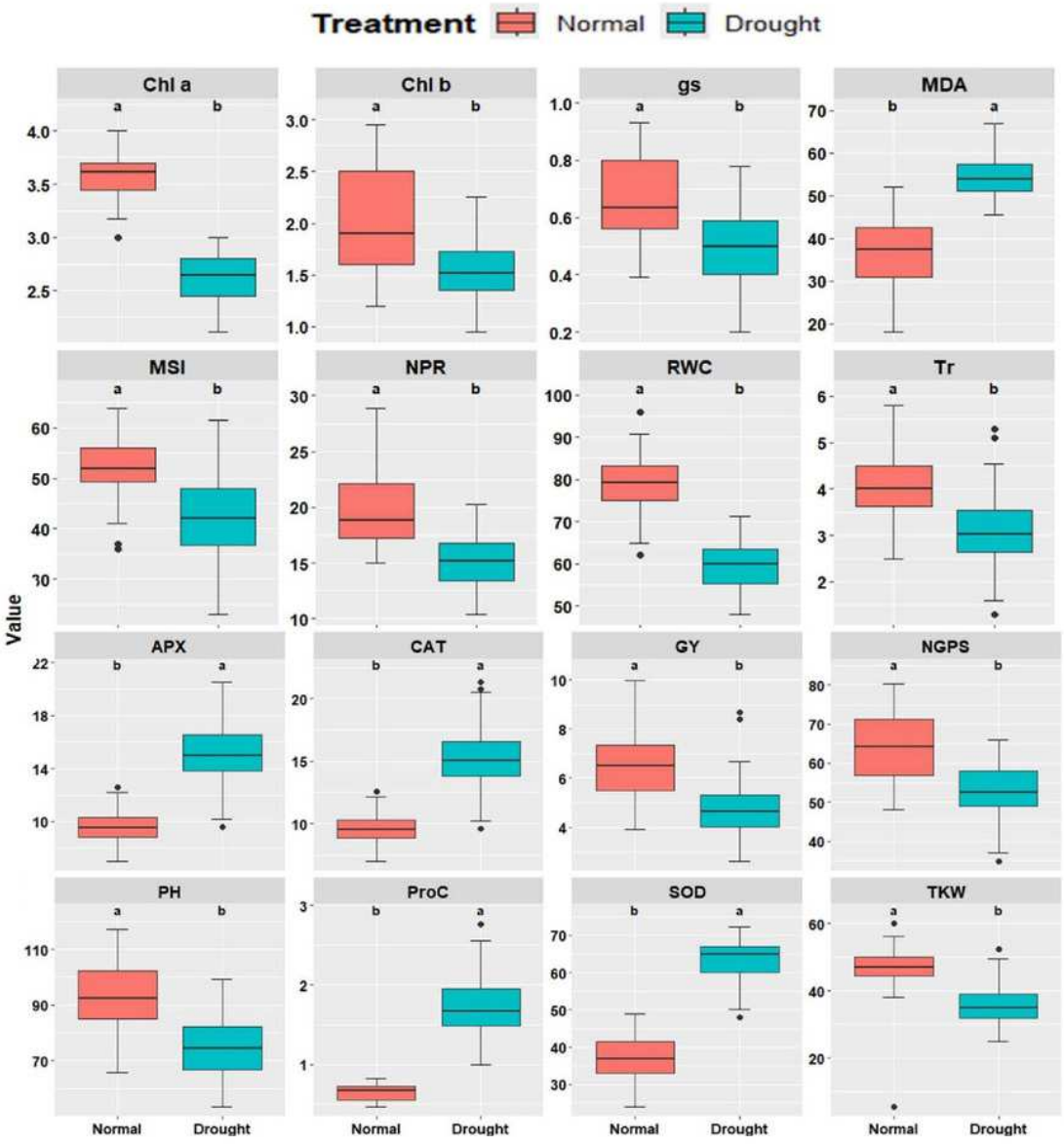

Figure 1. Comparative boxplots of 16 physiological and agronomic measured traits under normal and drought conditions. Chlorophyll *a* (mg/g FW), Chl*b*: Chlorophyll *b* (mg/g FW), NPR: Net photosynthetic rate (µmol $CO_2/m^2/s$, Tr: Transpiration rate (µmol $CO_2/m^2/s$), gs: Stomatal conductance (µmol $CO_2/m^2/s$), RWC: Relative water content (%), MSI: Membrane stability index (%), MDA: Malondialdehyde (µmol/g FW), Proc: Proline content (µmol/g DW), SOD: Superoxide dismutase (unit mg/ protein), CAT: Catalase (unit mg/ protein), APX: Ascorbate peroxidase (unit mg/ protein), PH: Plant height (cm), NGS: Number of grains /spike, TGW: 1000-grain weight (g), and GY: Grain yield (tons/ha).

Figure 2

Comparative performance of evaluated wheat genotypes

Comparative performance of evaluated wheat genotypes: (A): Chlorophyll a, (B): Chlorophyll b, (C): Net photosynthetic rate, (D): Transpiration rate, (E: Stomatal conductance, and (F): Relative water content. The standard error (SE) is shown by the bars above the columns.

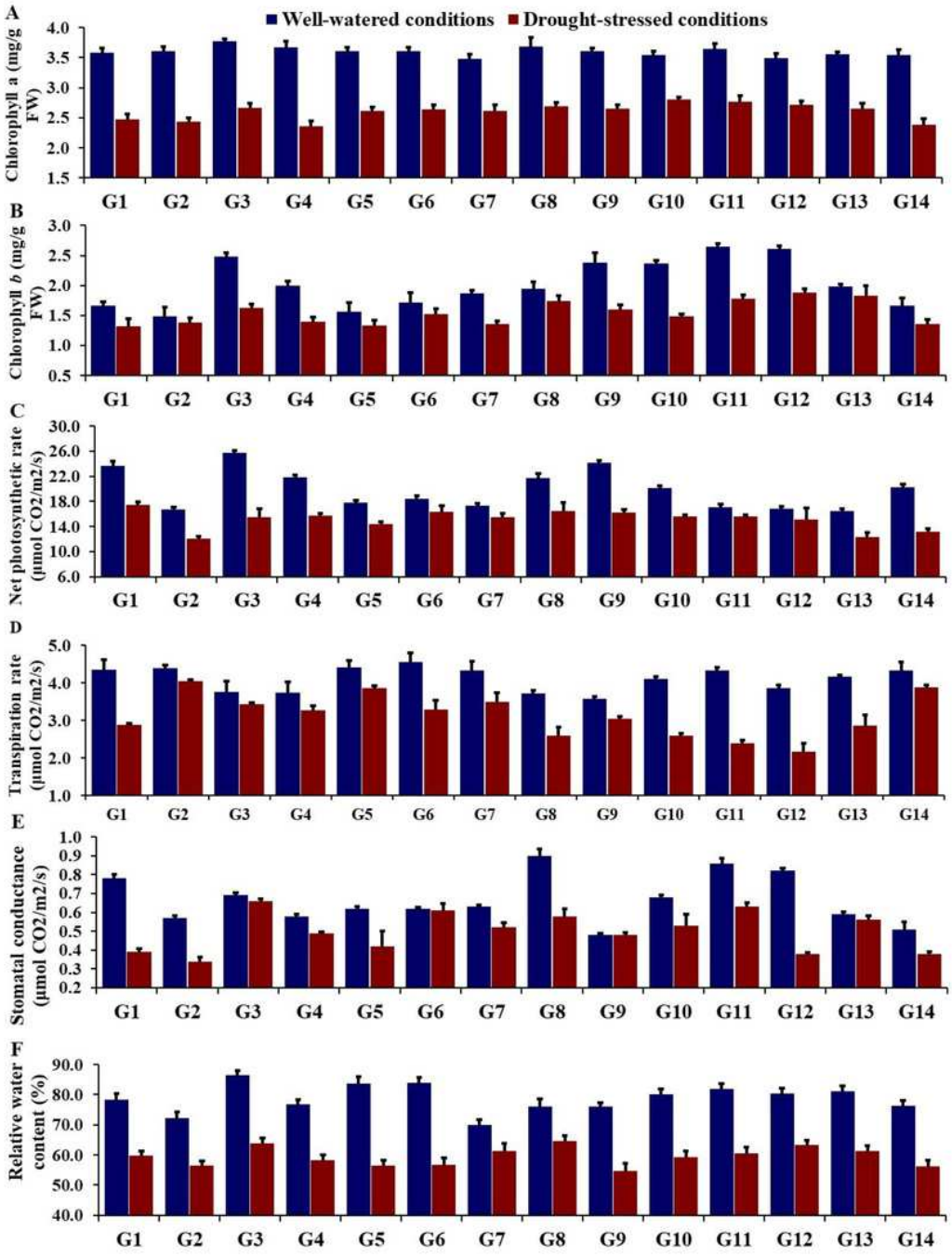

Figure 2. Comparative performance of evaluated wheat genotypes: (A): Chlorophyll *a*, (B): Chlorophyll *b*, (C): Net photosynthetic rate, (D): Transpiration rate, (E: Stomatal conductance, and (F): Relative water content. The standard error (SE) is shown by the bars above the columns.

Figure 3

Comparative performance of evaluated wheat genotypes

Comparative performance of evaluated wheat genotypes: (A): Membrane stability index, (B): Malondialdehyde, (C): Proline content, (D) Catalase, (E): Ascorbate peroxidase and (F): Superoxide dismutase. The standard error (SE) is shown by the bars above the columns

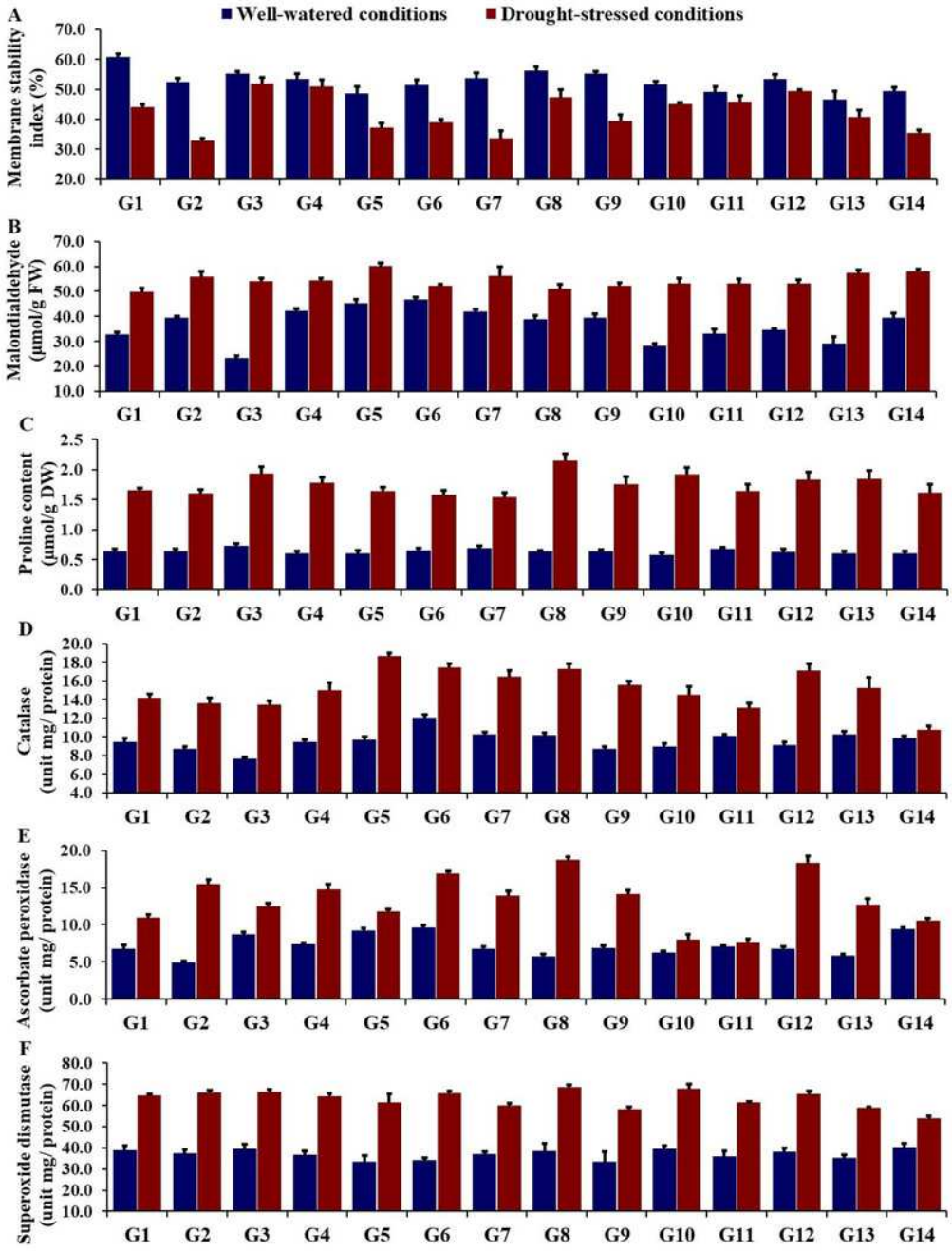

Figure 3. Comparative performance of evaluated wheat genotypes: (A): Membrane stability index, (B): Malondialdehyde, (C): Proline content, (D) Catalase, (E): Ascorbate peroxidase and (F): Superoxide dismutase. The standard error (SE) is shown by the bars above the columns.

Figure 4

Comparative performance of evaluated wheat genotypes

Comparative performance of evaluated wheat genotypes: (A): Plant height, (B): Number of grains/spike, (C): 1000-grain weight, and (D): Grain yield. The standard error (SE) is shown by the bars above the columns.

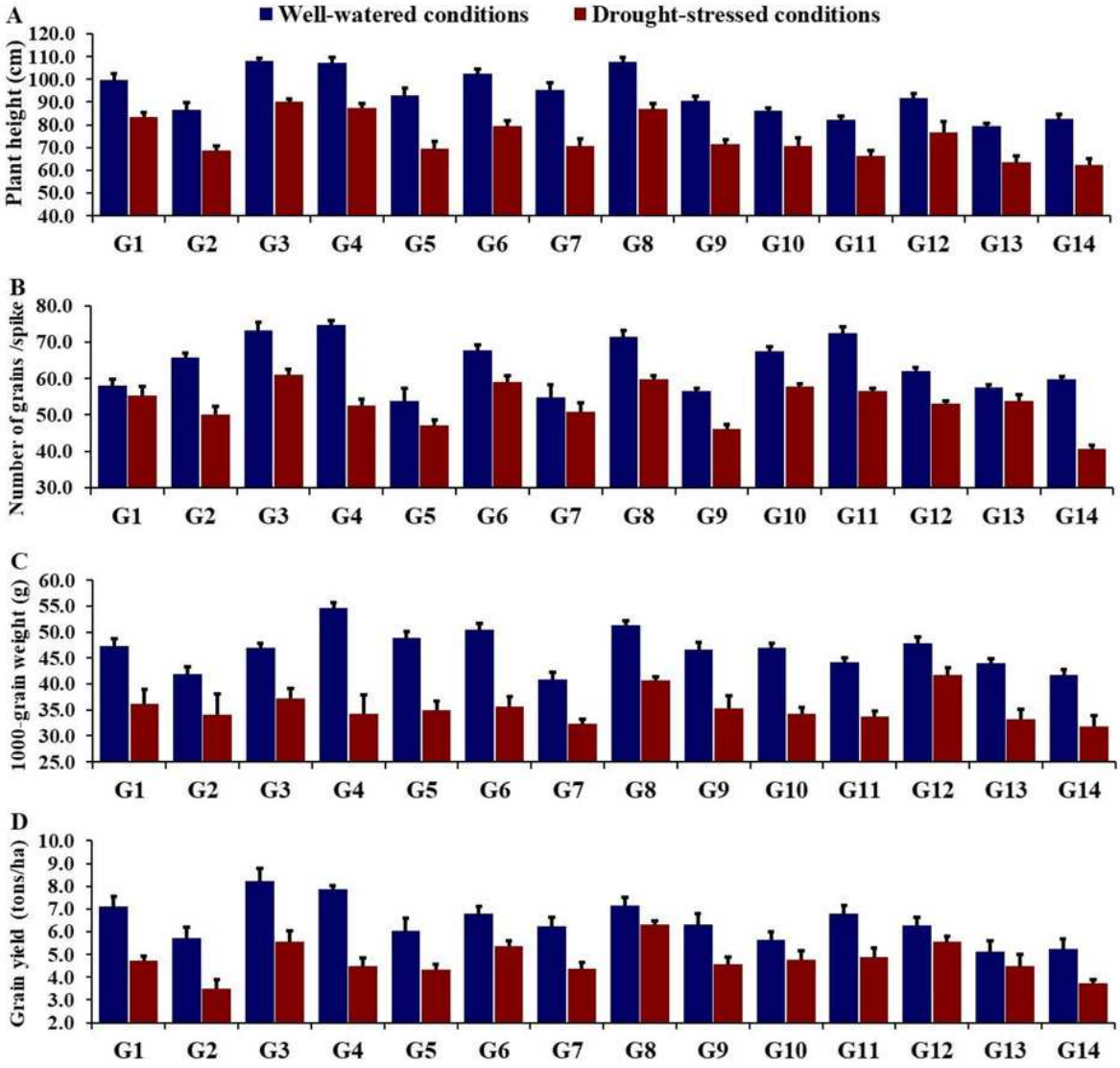

Figure 4. Comparative performance of evaluated wheat genotypes: (A): Plant height, (B): Number of grains/spike, (C): 1000-grain weight, and (D): Grain yield. The standard error (SE) is shown by the bars above the columns.

Figure 5

Heatmap for wheat genotypes based on studied physiological and agronomic traits under drought stress.

Heatmap for wheat genotypes based on studied physiological and agronomic traits under drought stress. Blue color indicates lower rank of studied character while red color indicates high rank. Chlorophyll a (mg/g FW), Chlb: Chlorophyll b (mg/g FW), NPR: Net photosynthetic rate (µmol CO2/m2/s, Tr: Transpiration rate (µmol CO2/m2/s), gs: Stomatal conductance (µmol CO2/m2/s), RWC: Relative water content (%), MSI: Membrane stability index (%), MDA: Malondialdehyde (µmol/g FW), Proc: Proline content (µmol/g DW), SOD: Superoxide dismutase (unit mg/ protein), CAT: Catalase (unit mg/ protein), APX: Ascorbate peroxidase (unit mg/ protein), PH: Plant height (cm), NGS: Number of grains /spike, TGW: 1000-grain weight (g), and GY: Grain yield (tons/ha).

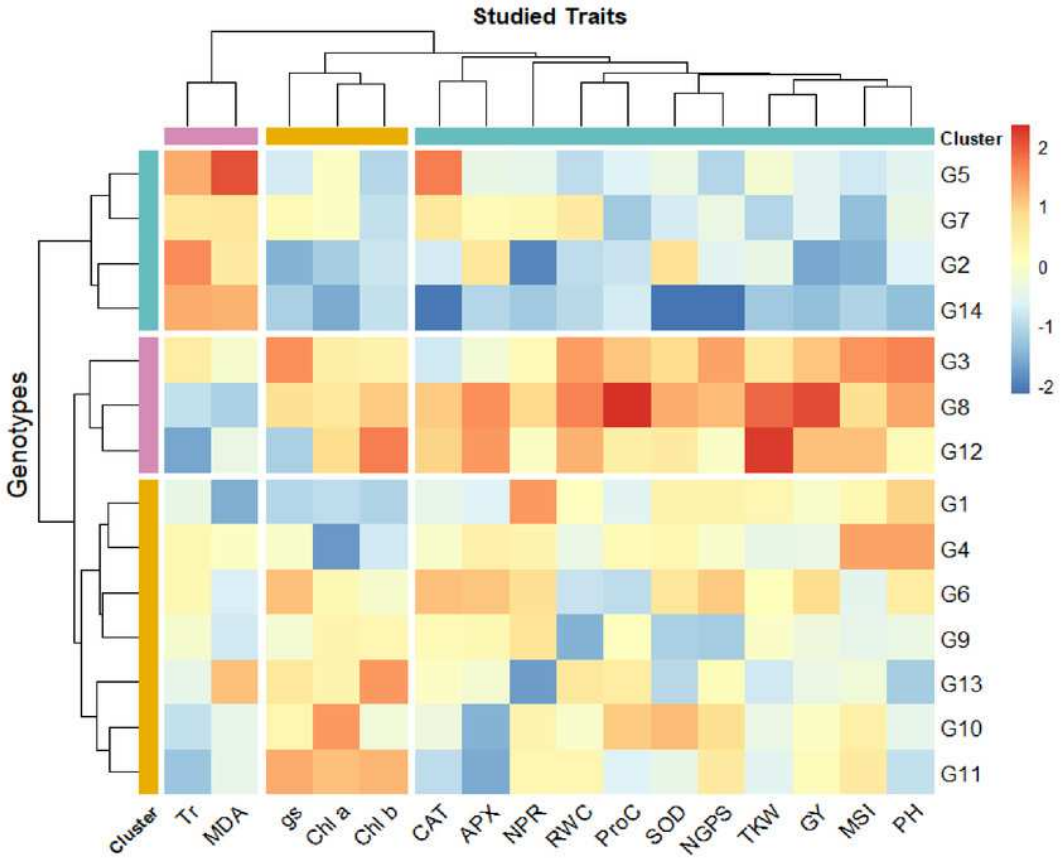

Figure 5. Heatmap for wheat genotypes based on studied physiological and agronomic traits under drought stress. Blue color indicates lower rank of studied character while red color indicates high rank. Chlorophyll *a* (mg/g FW), Chl*b*: Chlorophyll *b* (mg/g FW), NPR: Net photosynthetic rate (µmol $CO_2/m^2/s$, Tr: Transpiration rate (µmol $CO_2/m^2/s$), gs: Stomatal conductance (µmol $CO_2/m^2/s$), RWC: Relative water content (%), MSI: Membrane stability index (%), MDA: Malondialdehyde (µmol/g FW), Proc: Proline content (µmol/g DW), SOD: Superoxide dismutase (unit mg/ protein), CAT: Catalase (unit mg/ protein), APX: Ascorbate peroxidase (unit mg/ protein), PH: Plant height (cm), NGS: Number of grains /spike, TGW: 1000-grain weight (g), and GY: Grain yield (tons/ha).

Figure 6

Dendrogram depicting distances among fourteen wheat genotypes according to tolerance indices

Dendrogram depicting distances among fourteen wheat genotypes according to tolerance indices

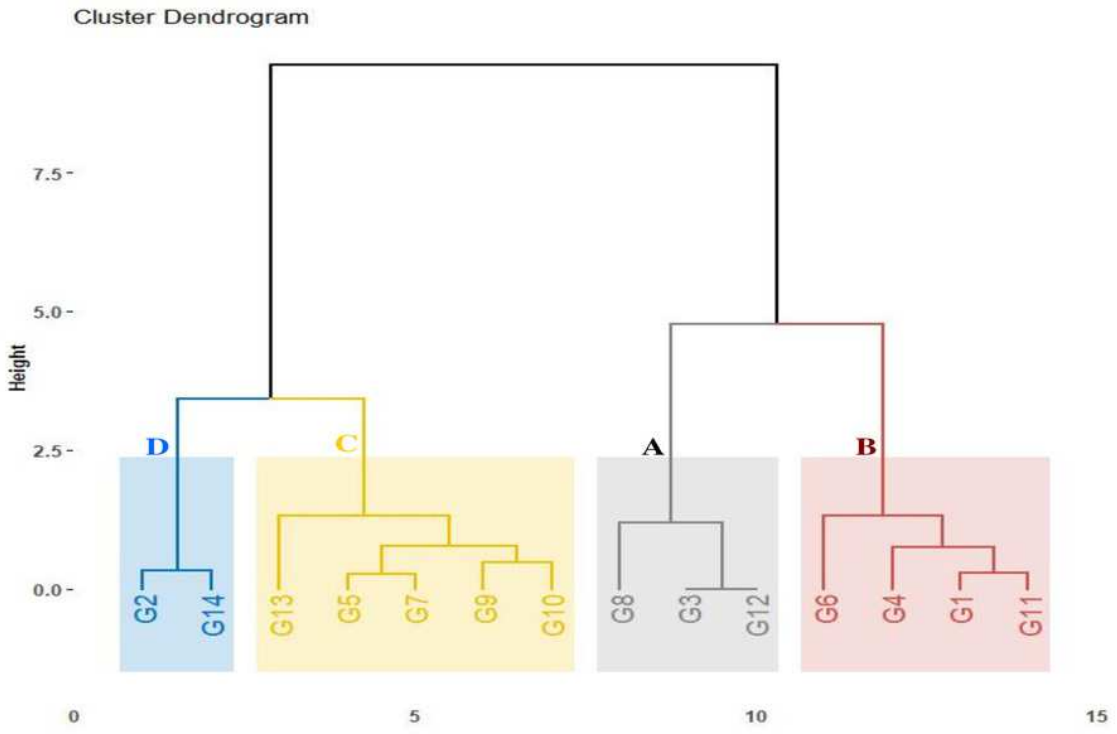

Figure 6. Dendrogram depicting distances among fourteen wheat genotypes according to tolerance indices.

Figure 7

Comparison of fourteen wheat genotype performance (G1-G14) and stability across environments using ranking and AMMI biplots for grain yield

Comparison of fourteen wheat genotype performance (G1-G14) and stability across environments using ranking and AMMI biplots for grain yield. E1: Normal conditions during the first season, E2: Drought conditions during the first season, E3: Normal condition durinf the second Season, and E4: Drought condition during second season.

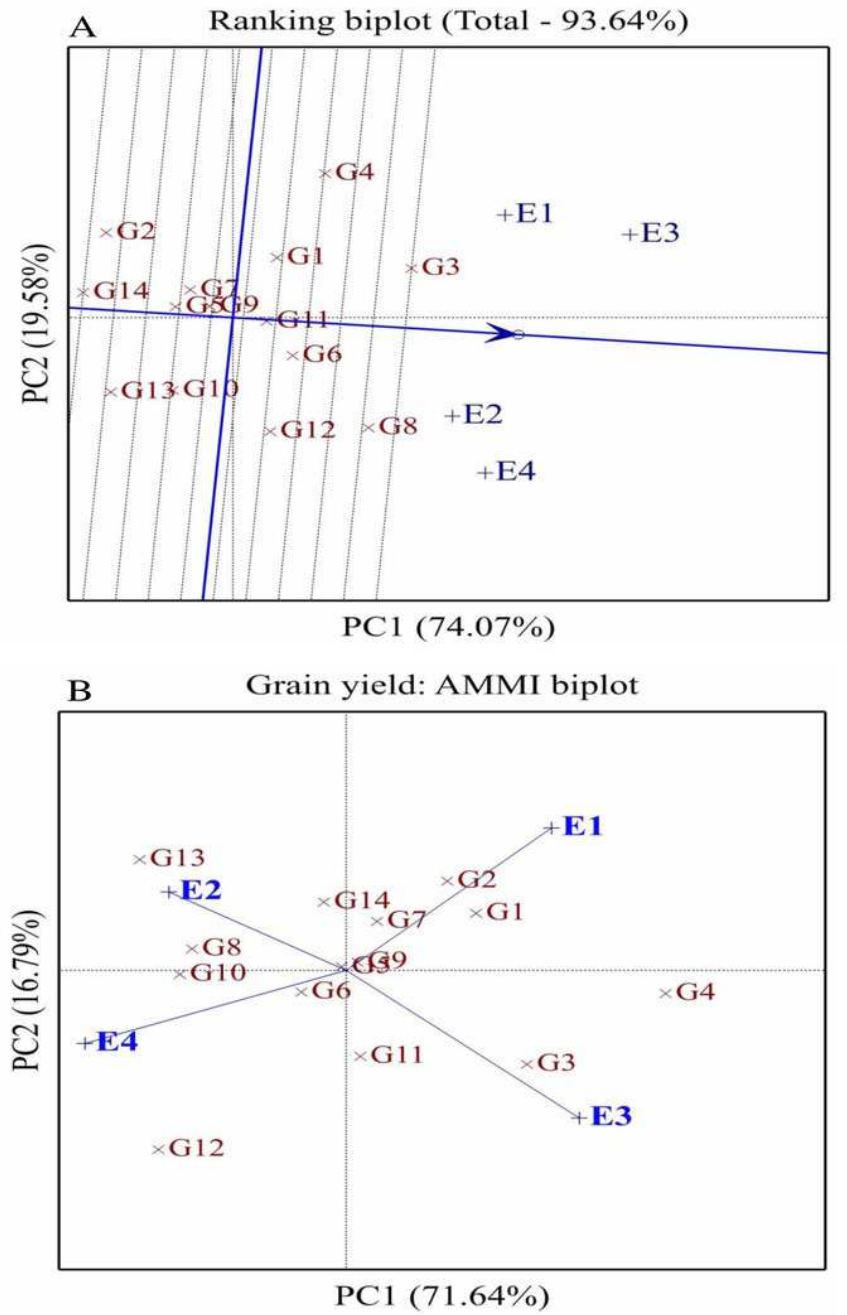

Figure 7. Comparison of fourteen wheat genotype performance (G1-G14) and stability across environments using ranking and AMMI biplots for grain yield. E1: Normal conditions during the first season, E2: Drought conditions during the first season, E3: Normal condition durinf the second Season, and E4: Drought condition during second season.

Figure 8

PCA biplot for studied physiological and agronomic in fourteen wheat genotypes under normal and water deficit conditions

PCA biplot for studied physiological and agronomic in fourteen wheat genotypes under normal and water deficit conditions (A), bar chart with contribution percentage of principal components to overall variance (B), and bar charts of trait contribution with dashed line of significant contribution (C and D).

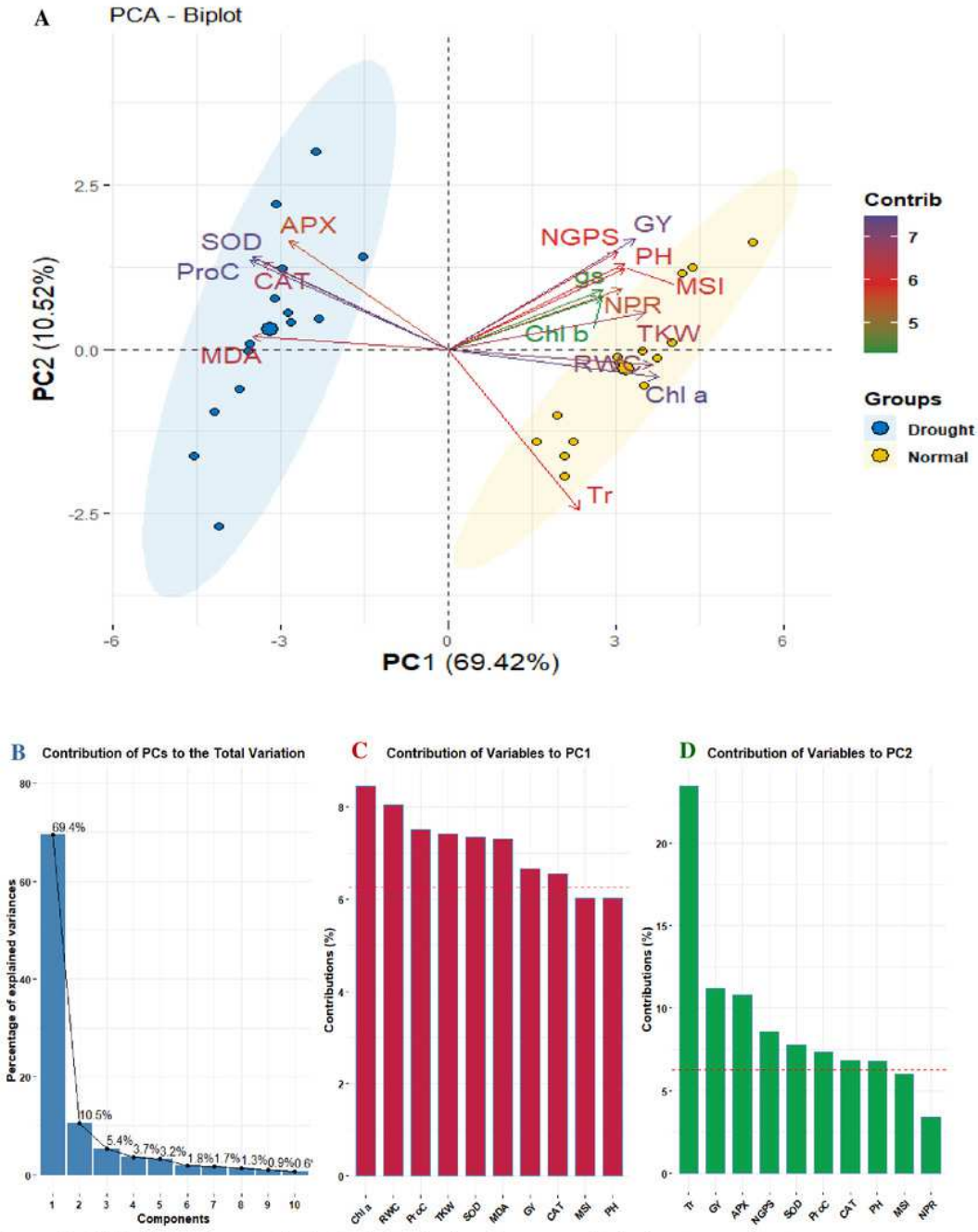

Figure 8. PCA biplot for studied physiological and agronomic in fourteen wheat genotypes under normal and water deficit conditions (A), bar chart with contribution percentage of principal components to overall variance (B), and bar charts of trait contribution with dashed line of significant contribution (C and D).

Figure 9

Matrix of Spearman correlation for physiological and agronomic characters under well-watered (A) and water deficit conditions (B).

Matrix of Spearman correlation for physiological and agronomic characters under well-watered (A) and water deficit conditions (B).

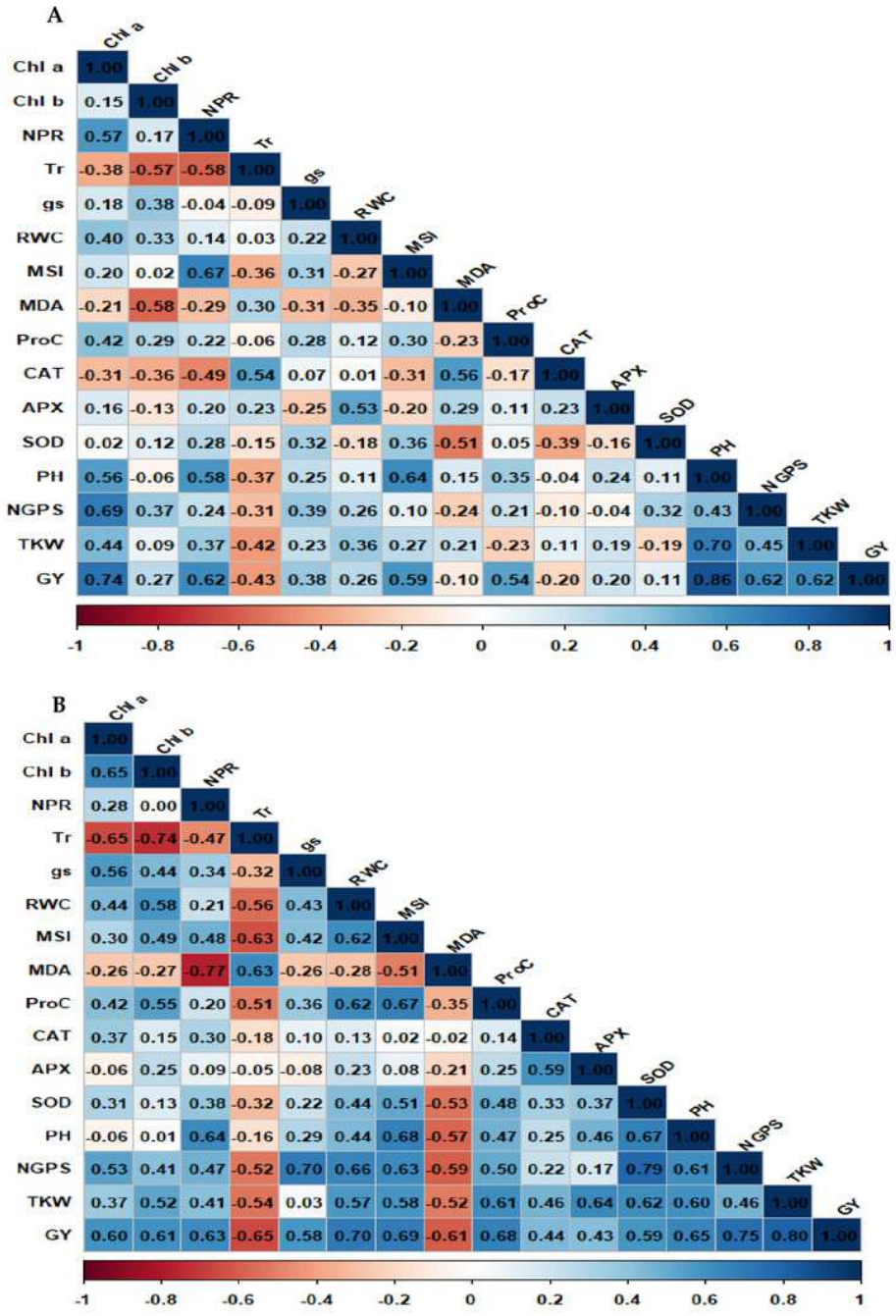

Figure 9. Matrix of Spearman correlation for physiological and agronomic characters under well-watered (A) and water deficit conditions (B).

Figure 10

Graphical Abstract

Graphical Abstract

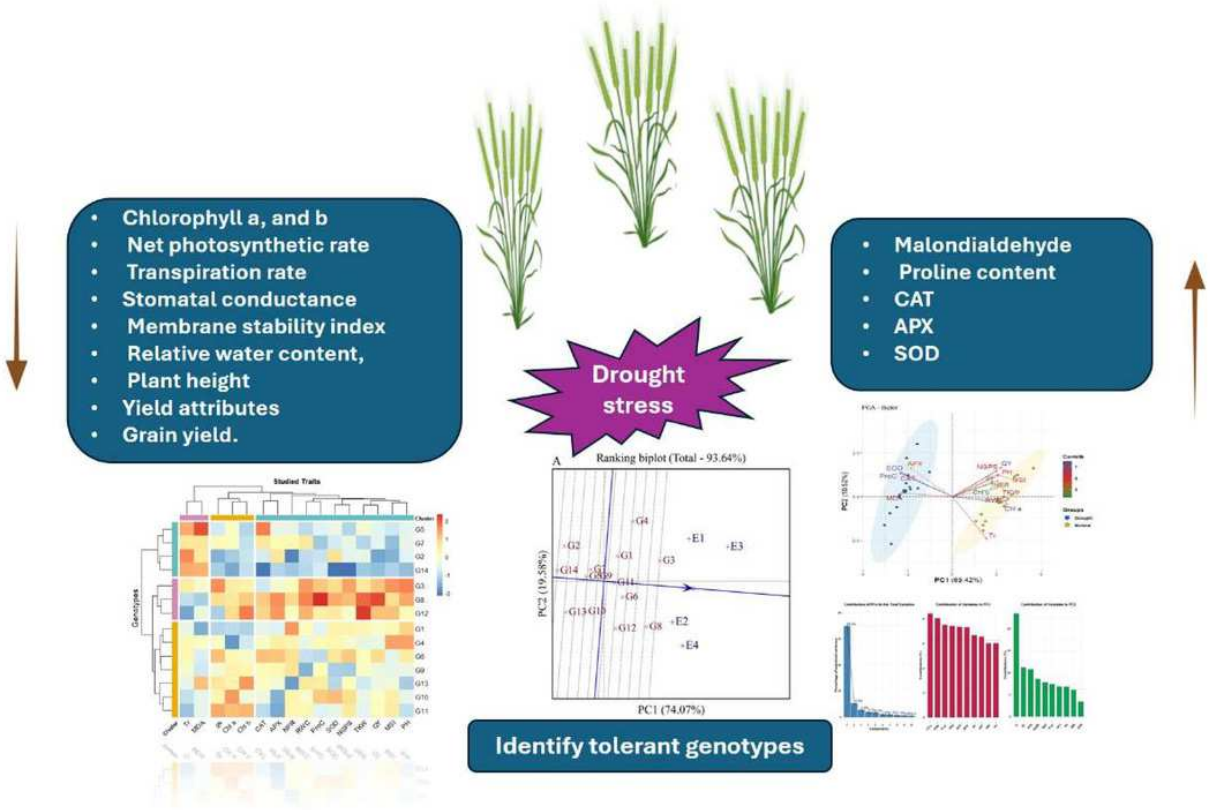
Chlorophyll a, and b
Net photosynthetic rate
Transpiration rate
Stomatal conductance
Membrane stability index
Relative water content,
Plant height
Yield attributes
Grain yield.
Malondialdehyde
Proline content
CAT
APX
SOD
Drought stress
Studied Traits
Genotypes
A
Ranking biplot (Total - 93.64%)
PC2 (19.58%)
PC1 (74.07%)
Identify tolerant genotypes

Table 1(on next page)

Analysis of variance (mean squares) for evaluated characters of the assessed genotypes under well watered and drought stress conditions over the two seasons of 2021/2022 and 2022/2023.

Analysis of variance (mean squares) for evaluated characters of the assessed genotypes under well watered and drought stress conditions over the two seasons of 2021/2022 and 2022/2023.

Table 1. Analysis of variance (mean squares) for evaluated characters of the assessed genotypes under well watered and drought stress conditions over the two seasons of 2021/2022 and 2022/2023.

Source of Variance	df	Chl*a* §	Chl*b*	NPR	Tr	gs	RWC	MSI	MDA
Growing season (Gs)	1	1.15*	0.74	24.18	1.54	0.17*	780.2	411.3	692.6
Replication/(Gs)	4	0.130	0.157	9.69	0.79	0.02	67.18	96.94	95.61
Irrigation (Ir)	1	41.38**	9.89**	947.0**	40.80**	1.19**	15676**	4492**	13021**
Ir×GS	1	0.57	0.58	12.39	0.85*	0.001	72.32	13.20	22.84
Error a	4	0.29	0.11	3.43	0.09	0.001	59.86	21.10	4.58
Genotypes (Gen)	13	0.07**	0.93**	53.03**	1.72**	0.10**	106.9**	204.7**	191.1**
Gen×Gs	13	0.03*	0.28**	12.47**	2.04**	0.02**	14.02	69.17**	15.07**
Gen×Ir	13	0.08**	0.26**	20.59**	0.91**	0.06**	77.79**	118.8**	138.8**
Gen×Gs×Ir	13	0.04**	0.30**	6.22**	1.14**	0.01**	15.73	81.55**	24.05**
Error b	104	0.01	0.01	0.50	0.10	0.001	12.35	9.74	4.54
Source of Variance	**df**	**Proc**	**CAT**	**APX**	**SOD**	**PH**	**NGS**	**TGW**	**GY**
Growing season (Gs)	1	0.97	36.79**	38.96*	1318**	407.2	40.43	746.9**	4.74*
Replication/(Gs)	4	0.34	1.52	2.135	3.62	185.2	5.323	8.92	0.517
Irrigation (Ir)	1	51.52**	1301**	1519**	28529**	15016**	4939**	5332**	123.2**
Ir×GS	1	4.16*	47.58**	44.95**	312.2*	51.19	53.72*	281.9	34.63*
Error a	4	0.33	0.55	0.51	16.08	306.6	3.10	101.9	2.05
Genotypes (Gen)	13	0.09**	20.96**	37.80**	84.74**	1077**	419.8**	103.1**	6.54**
Gen×Gs	13	0.04**	0.72	10.71**	44.71**	54.78**	41.94**	66.94**	0.45
Gen×Ir	13	0.10**	11.85**	47.91**	55.97**	28.73*	101.0**	36.05**	1.91**
Gen×Gs×Ir	13	0.04**	1.03	7.68**	33.82**	40.58**	52.74**	13.24	0.35
Error b	104	0.001	0.70	1.54	5.83	15.68	11.38	14.63	0.80

§ Chl*a:* Chlorophyll *a* (mg/g FW), Chl*b*: Chlorophyll *b* (mg/g FW), NPR: Net photosynthetic rate (µmol $CO_2/m^2/s$, Tr: Transpiration rate (µmol $CO_2/m^2/s$), gs: Stomatal conductance (µmol $CO_2/m^2/s$), RWC: Relative water content (%), MSI: Membrane stability index (%), MDA: Malondialdehyde (µmol/g FW), Proc: Proline content (µmol/g DW), SOD: Superoxide dismutase (unit mg/ protein), CAT: Catalase (unit mg/ protein), APX: Ascorbate peroxidase (unit mg/ protein), NGS: Number of grains /spike, PH: Plant height (cm), TGW: 1000-grain weight (g), and GY: Grain yield (tons/ha).

* and ** indicate p-value < 0.05 and 0.01 in the same order.

Table 2(on next page)

G enetic variability parameters for physiological and agronomic characters in evaluated genotypes under normal (NOR) and water deficit (DRO) conditions

G enetic variability parameters for physiological and agronomic characters in evaluated genotypes under normal (NOR) and water deficit (DRO) conditions

Table 2. Genetic variability parameters for physiological and agronomic characters in evaluated genotypes under normal (NOR) and water deficit (DRO) conditions.

Parameter	Irrigation	Chl *a*	Chl *b*	NPR	Tr	gs	RWC	MSI	MDA
σ^2g	Nor	0.01	0.15	9.62	0.08	0.02	19.11	12.22	46.04
	Dro	0.02	0.04	2.49	0.32	0.01	7.58	38.45	7.43
σ^2p	Nor	0.02	0.18	10.28	0.21	0.02	31.23	20.50	51.42
	Dro	0.03	0.05	2.82	0.41	0.01	20.16	49.66	11.13
GCV	Nor	1.54	19.40	15.63	7.03	19.27	5.55	6.63	18.47
	Dro	5.19	12.70	10.44	18.13	20.52	4.63	14.64	5.02
PCV	Nor	4.06	20.72	16.15	11.02	19.63	7.09	8.59	19.52
	Dro	6.19	13.89	11.12	20.46	22.37	7.55	16.64	6.14
H^2b	Nor	14.46	87.73	93.61	40.63	96.32	61.18	59.63	89.52
	Dro	70.25	83.68	88.08	78.55	84.14	37.60	77.42	66.76
GA	Nor	0.04	0.76	6.18	0.38	0.26	7.04	5.56	13.22
	Dro	0.23	0.37	3.05	1.03	0.19	3.48	11.24	4.59
Parameter	**Irrigation**	**Proc**	**CAT**	**APX**	**SOD**	**PH**	**NGPS**	**TKW**	**GY**
σ^2g	Nor	0.01	0.95	2.11	4.29	96.25	51.91	10.71	0.70
	Dro	0.03	4.28	11.66	17.23	82.91	31.04	7.59	0.44
σ^2p	Nor	0.02	1.47	2.39	10.32	116.58	62.56	37.65	1.58
	Dro	0.03	5.17	14.46	22.84	93.96	43.14	9.91	1.16
GCV	Nor	6.17	10.16	20.06	5.59	10.47	11.26	7.01	12.93
	Dro	9.66	13.63	25.75	6.58	12.17	10.49	7.78	13.99
PCV	Nor	6.83	12.60	21.31	8.68	11.52	12.36	13.15	19.44
	Dro	10.02	14.98	28.67	7.58	12.96	12.36	8.89	22.61
H^2b	Nor	81.54	65.05	88.58	41.53	82.56	82.98	28.46	44.25
	Dro	92.98	82.81	80.61	75.41	88.24	71.95	76.60	38.30
GA	Nor	0.07	1.62	2.82	2.75	18.36	13.52	3.60	1.15
	Dro	0.34	3.88	6.32	7.43	17.62	9.74	4.97	0.85

σ^2p: Phenotypic variance, GCV: genotypic coefficient of variation, σ^2g: Genotypic variance, PCV: phenotypic coefficient of variation, H^2b: Broad-sense heritability, GA: Genetic Advance.