



Wheat Genotypes with Higher Intercellular CO₂ Concentration, Rate of Photosynthesis, and Antioxidant Potential Can Better Tolerate Drought Stress

Aman Ullah¹ · Rashid Said Al-Rajhi¹ · Abdullah M. Al-Sadi¹ · Muhammad Farooq¹

Received: 29 March 2021 / Accepted: 3 June 2021 / Published online: 21 June 2021
© Sociedad Chilena de la Ciencia del Suelo 2021

Abstract

This study was conducted to identify physiological and morphological traits linked with drought tolerance and variation among different bread wheat genotypes. In this study, 20 different bread and durum wheat genotypes were characterized on morphological, biochemical, and physiological traits under well-watered (70% water holding capacity; WHC) and drought stress (35% WHC) conditions. Drought stress significantly affected different morphological, biochemical, and physiological traits of wheat; however, significant genotypic differences were noted in both bread and durum wheat genotypes. Under drought stress, the activities of catalase and superoxide dismutase were higher in all tested genotypes compared to well-watered conditions. Regarding physiological traits, an overall increase in the leaf temperature, SPAD chlorophyll contents, intercellular CO₂ concentration (C_i), water use efficiency (WUE), intrinsic (WUE_i), and instantaneous (WUE_{ins}) water use efficiencies was recorded across the genotypes under drought stress compared to well-watered conditions. The principal component analysis (PCA) showed that under drought stress, the two principal components (PCs) had a significant variation for morphological and biochemical traits and the contribution of variation was 39.1 and 25.6% by PC1 and PC2, respectively. For physiological traits, under drought, the PC1 contributed 59.2 and PC2 18.4% of the total variability and the C_i, SPAD chlorophyll contents, photosynthesis (A), WUE, WUE_i, and WUE_{ins} were negatively correlated, while stomatal conductance (g_s), transpiration rate (T), and the ratio of A/C_i were positively correlated with PC1. The genotypes SQU-97, SQU-95, and SQU-80 were grouped based on high C_i, A, WUE, WUE_i, and WUE_{ins}, while genotypes SQU-85, SQU-86, SQU-89, and SQU-90 were grouped based on high g_s, T, and A/C_i. The agglomerative hierarchical clustering clustered the studied genotypes into three classes and the variation within the classes was 47.6%, while between the classes was 52.4%. The wheat genotypes (SQU-79, SQU-82, SQU-87, SQU-89, SQU-91, SQU-93, SQU-94, SQU-95, SQU-96, and SQU-98) with higher SPAD chlorophyll contents, antioxidant activities, intercellular CO₂ concentration, water use efficiencies, and net photosynthesis were better able to tolerate the drought stress.

Keywords Gas exchange · Water relations · Catalase · Superoxide dismutase

1 Introduction

Wheat (*Triticum aestivum* L.) is a staple for more than 2.5 billion people worldwide (www.wheatatlas.org) and is the largest rain-fed grain crop. However, water scarcity and drought are serious threats to sustainable wheat production.

About two-thirds of the global wheat-growing areas may face “severe, prolonged and near-simultaneous droughts” by the end of this century (Trnka et al. 2019). This challenge and the increasing population worldwide require developing strategies for sustainable wheat production for global food security (Farooq et al. 2022).

Drought stress suppresses plant growth and yield formation through alteration in plant water relations and nutrient uptake, and reduction in the rate of net photosynthesis and assimilates translocation (Farooq et al. 2009; Ullah and Farooq 2021). However, the extent of growth suppression and yield reduction depends on the severity and duration of the drought stress (Farooq et al. 2009). Drought also causes a

✉ Muhammad Farooq
farooqcp@squ.edu.om

¹ Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, AlKhouf 123, Muscat, Oman

significant decrease in the activities of key enzymes involved in carboxylation, grain development, and other metabolic events (Zahra et al. 2021). The drought-induced decrease in photosynthesis causes an imbalance between the excitation and utilization of electrons and results in the over-production of reactive oxygen species (ROS) (Reddy et al. 2004; Fang et al. 2015). These ROS, including superoxide anions (O_2^-), singlet oxygen (1O_2), hydroxyl radicals (OH \cdot), and hydrogen peroxide (H_2O_2), cause oxidative damages to the biological membranes, nucleic acids, and proteins (Farooq et al. 2019).

The ability of plants to sustain growth, drought tolerance, involves several morphological, physiological, biochemical, and molecular modifications at cellular tissue, organ, and organism levels. These modifications include activation of antioxidant defense systems (involving both enzymatic and non-enzymatic components) (Anjum et al. 2016), increases of water uptake by developing deep root systems, reduction in water loss through a decrease in stomatal density and conductance, and accumulation of osmolytes (Chipilski et al. 2012; Huseynova, 2012; Abid 2016). These modifications help plants grow and produce a fair amount of yield even under drought (Izanloo et al. 2008; Farooq et al. 2014).

The activation of plant antioxidant defense systems helps to scavenge the ROS and protects the plants from oxidative stress (Farooq et al. 2017). The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), ascorbic acid peroxidase (APX), and dehydroascorbic acid reductase (Reddy et al. 2004). As the first line of defense, SOD dismutates the superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2) (Cakmak and Horst 1991) whereas the other components of enzymatic antioxidant modulate to keep homeostasis of H_2O_2 concentration (Dröge 2002). However, for the scavenging of singlet oxygen (1O_2) and hydroxyl radicals (OH \cdot), non-enzymatic antioxidants are more effective (Bose et al. 2014). The non-enzymatic antioxidants consist of low-molecular-weight metabolites and include glutathione, tocopherols, ascorbic acid, and flavonoids (Noctor and Foyer 1998).

The accumulation of osmolytes including proline, glycine betaine, and trehalose helps maintain tissue water potential and thus allows to maintain physiological activities (Farooq et al. 2009, 2018). In addition to their role in osmotic adjustment, these osmolytes also help improve drought tolerance by restricting protein denaturation, acting as a free radical scavenger, and stabilizing biological membranes (Farooq et al. 2009, 2018).

Identification of key morphological, physiological, and biochemical adaptation under drought may help improving growth and yield formation under water deficit conditions (Bowne et al. 2012; Han et al. 2015). This necessitates exploring a wide group of wheat genotypes

for drought tolerance. The determination of diversity within and between the wheat populations is the basis for elucidation of genetic structure and improvement of quantitative traits including drought tolerance (Mwadzingeni et al. 2016). Although several studies (Ahmad et al. 2014; Bilal et al. 2015; Ahmed et al. 2019) are available on the response of wheat genotypes to drought, there is limited information on the morphological, biochemical, and physiological responses of a diverse group of bread and durum wheat genotypes to drought stress. This study hypothesized that wheat genotypes with higher intercellular CO_2 concentration, antioxidant potential, and the rate of photosynthesis can better tolerate drought stress. The specific objective of this study was to determine physiological and morpho-biochemical traits linked with drought tolerance and genotypic variation among different wheat genotypes.

2 Material and Methods

2.1 Experimental Site, Treatment Details, and Design

This experiment was conducted in the glasshouse at the Agricultural Experimental Station, Sultan Qaboos University, Oman, in 2020. The temperature in the growth chamber was maintained at $25/18 \pm 2$ °C day/night.

Twenty wheat genotypes: 5 collected from International Maize and Wheat Improvement Centre (CIMMYT) and 15 from International Centre for Agricultural Research in Dry Areas (ICARDA) (Table S1), were sown in plastic pots (1.5 kg) filled with Universal pot ground media (Van-Eganond potgrond, Amsterdam, Holland) maintained at 35% water holding capacity (WHC) (drought stress) and 70% WHC (control). Three seeds per pot were sown in all 120 pots (20 wheat genotypes \times 2 treatment levels \times 3 replications). Plants were raised under well-watered conditions for 2 weeks, on the 16th day after sowing drought was imposed by using the moisture probe meter. The characteristics of media used were organic matter (85–95%), electrical conductivity (0.6–1.5 mS/cm), pH (5.0–6.0), moisture contents (50%), carbon into nitrogen ratio of (50:1), and sodium chloride < 1%. The experiment was conducted under a completely randomized design in a factorial arrangement with three repeats. There were two pots on each replication. The experiment was harvested on the 31st day after sowing. Gas exchange parameters, SPAD values, and the relative leaf water contents (RLWC) were recorded on the 30th day after sowing whereas the rest of the observations were recorded at harvest.

2.2 2.2. Observations.

2.2.1 Shoot Length (cm) and Seedling Dry Weight (g)

Shoot length (2 plants per pot) was measured from the base to the top of the plants with a measuring ruler at the harvesting. The seedling dry weight (shoots only) (2 plants per pot) was determined by drying the seedling in an electric oven until constant weight.

2.2.2 Relative Leaf Water Contents (%)

To determine the relative leaf water contents (RLWC), fresh leaves (W_f) were weighed and then soaked in water for 4 h to take the saturated weight (W_s). The leaves were then oven-dried at 80 °C until constant weight and then dry weight (W_d) was recorded. The RLWC was calculated as described by Barr and Weatherley (1962).

$$\text{RLWC} = (W_f - W_d) / (W_s - W_d) \times 100$$

2.2.3 Biochemical Traits

The catalase activity was determined using the method of Chance and Maehly (1955). Briefly, leaf samples (1 g) were digested in a 3-mL solution, which comprised of phosphate buffer and H_2O_2 . The changes in absorbance of the reaction solution were recorded after every 20 s at 240 nm. The SOD activity was estimated following the method of Gianopolitis and Ries (1977). In this method, the inhibition of photochemical reduction of *p*-nitro blue tetrazolium chloride (NBT) was noted with the spectrophotometric measurement at 560 nm. The reaction mixture contained 2.9 ml K-P buffer, 0.5 mL 50 mM Na_2CO_3 , 0.5 mL 50 mM L-methionine, 0.5 mL 75 μM NBT, and 500 μL 2 μM riboflavin with 100 μL supernatants. After the addition of riboflavin, the tubes containing the reaction mixture were placed under the light in a growth room for 8 min, and then absorbance was recorded at 560 nm.

2.2.4 Physiological Traits

The SPAD value of chlorophyll (2 plants per pot) was taken from the penultimate leaf using the SPAD meter (SPAD-502Plus; Konica Minolta, Osaka, Japan). Different gas exchange traits as stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1}$), net CO_2 assimilation rate (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), intercellular CO_2 concentration (C_i ; $\mu\text{mol m}^{-1}$), the ratio of CO_2 net assimilation rate (A) and intercellular CO_2 concentration (C_i) (A/C_i), transpiration rate (T ; $\text{mmol m}^{-2} \text{s}^{-1}$), water use efficiency (WUE), and leaf temperature (°C) were

measured by using the CIRAS-3 portable photosynthesis system (Amesbury, MA 01,913, USA, 2019 with leaf area fixed at $= 1.75 \text{ cm}^{-2}$, cuvette temperature set to 30 °C). The intrinsic water use efficiency (ratio of A and g_s) (WUE_i) and instantaneous water use efficiency (ratio of A/T) (WUE_{ins}) were calculated following Martin and Ruiz-Torres (1992) and Anyia and Herzog (2004), respectively. The external leaf CO_2 concentration (C_a) and artificial saturating photosynthetic active radiation (PAR) were fixed at 400 $\mu\text{mol mol}^{-1}$ and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The relative humidity was maintained at 45% and the flow rate of water at 500 μm along with a vapor pressure deficit of 1.7 kPa in the cuvette to prevent the stomatal closure. The readings were taken on the 15th day of drought stress from two plants per pot with three biological replicates on a clear and cloudless day. Measurements were taken in the morning between 08.00 a.m. and 11.0 a.m. from the penultimate leaf by clamping the leaf inside the sensor head.

2.3 Data Analysis

The data were analyzed using analysis of variance technique under a completely randomized design with factorial arrangement with the aid of XLSTAT-2020. The principal component analysis (PCA) was performed using XLSTAT-2020 to determine the multivariate ordination of physiological and morphological traits of tested wheat genotypes. The PCA plots were constructed using XLSTAT-2020 (Addinsoft-2020; XLSTAT statistical and data analysis solution; New York, USA; <https://www.xlstat.com>). Agglomerative hierarchical clustering (AHC) of genotypes was done on physiological traits under drought stress and the dendrogram was constructed with XLSTAT-2020 on dissimilarity (Euclidean distance) using Ward's method.

3 Results

3.1 3.1 Growth Traits

Analysis of variance showed that drought stress significantly affected the growth traits (shoot length, relative leaf water contents, and seedling dry weight) of wheat genotypes (Table 1). The individual effects of drought stress, genotypes, and drought stress \times genotypes were significant ($p \leq 0.05$) for all growth attributes (Table 1). The fitness of the model and R square of the fitness of a model had good significance for different studied growth parameters (Tables 2S–4S).

There was a substantial genotypic difference among the wheat genotypes regarding different studied growth traits under drought stress and well-watered conditions (Fig. 1). Under drought stress, all the studied growth traits were

Table 1 Analysis of variance for different morpho-biochemical and physiological traits of wheat genotypes under well-watered and drought stress conditions

Source	DF	Mean squares	F	Pr>F	Mean squares	F	Pr>F	Mean squares	F	Pr>F	
Drought stress (DS)	1	Shoot length	99.734	< 0.0001	Relative leaf water contents	2478.032	< 0.0001	Seedling dry weight	2.420	133.692	< 0.0001
Genotypes (G)	19	146.192	55.653	< 0.0001	5207.419	71.409	< 0.0001	0.442	24.449	< 0.0001	
DS × G	19	81.578	3.685	< 0.0001	150.061	45.576	< 0.0001	0.080	4.432	< 0.0001	
		5.402			95.774						
		Catalase contents			Superoxide dismutase contents						
Drought stress (DS)	1	312.992	< 0.0001	< 0.0001	10,464.299	310.636	< 0.0001				
Genotypes (G)	19	32.604	< 0.0001	< 0.0001	1036.765	30.777	< 0.0001				
DS × G	19	1.607	0.075		712.953	21.164	< 0.0001				
		Leaf temperature			SPAD chlor						
Drought stress (DS)	1	58.800	123.648	< 0.0001	17.672	10.379	0.002	45,649.502	255.742	< 0.0001	
Genotypes (G)	19	1.072	2.255	0.006	97.518	57.277	< 0.0001	2609.102	14.617	< 0.0001	
DS × G	19	1.667	3.505	< 0.0001	14.093	8.277	< 0.0001	982.072	5.502	< 0.0001	
		g_s			A			T			
Drought stress (DS)	1	0.379	5428.024	< 0.0001	228.942	187.744	< 0.0001	279.487	571.617	< 0.0001	
Genotypes (G)	19	0.010	141.249	< 0.0001	10.088	8.273	< 0.0001	9.424	19.274	< 0.0001	
DS × G	19	0.008	117.738	< 0.0001	8.224	6.744	< 0.0001	7.973	16.306	< 0.0001	
		A/Ci			WUE			WUEi			
Drought stress (DS)	1	0.006	298.391	< 0.0001	79.243	885.046	< 0.0001	198,019.410	1114.188	< 0.0001	
Genotypes (G)	19	0.000	4.677	< 0.0001	8.763	97.871	< 0.0001	15,413.864	86.729	< 0.0001	
DS × G	19	0.000	3.064	0.000	8.005	89.403	< 0.0001	12,482.259	70.233	< 0.0001	
		WUEins									
Drought stress (DS)	1	62.322	372.430	< 0.0001							
Genotypes (G)	19	8.333	49.797	< 0.0001							
DS × G	19	6.931	41.418	< 0.0001							

df degree of freedom, *P* < 0.05, *Ltemp* leaf temperature, *SPAD chlor* SPAD chlorophyll contents, *Ci* intercellular CO₂ concentration, *gs* stomatal conductance, *A* net photosynthetic rate, *T* transpiration rate, *A/Ci* ratio of photosynthetic rate or net CO₂ assimilation rate and intercellular CO₂ concentration, *WUE* water use efficiency, *WUEi* intrinsic water use efficiency, *WUEins* instantaneous water use efficiency

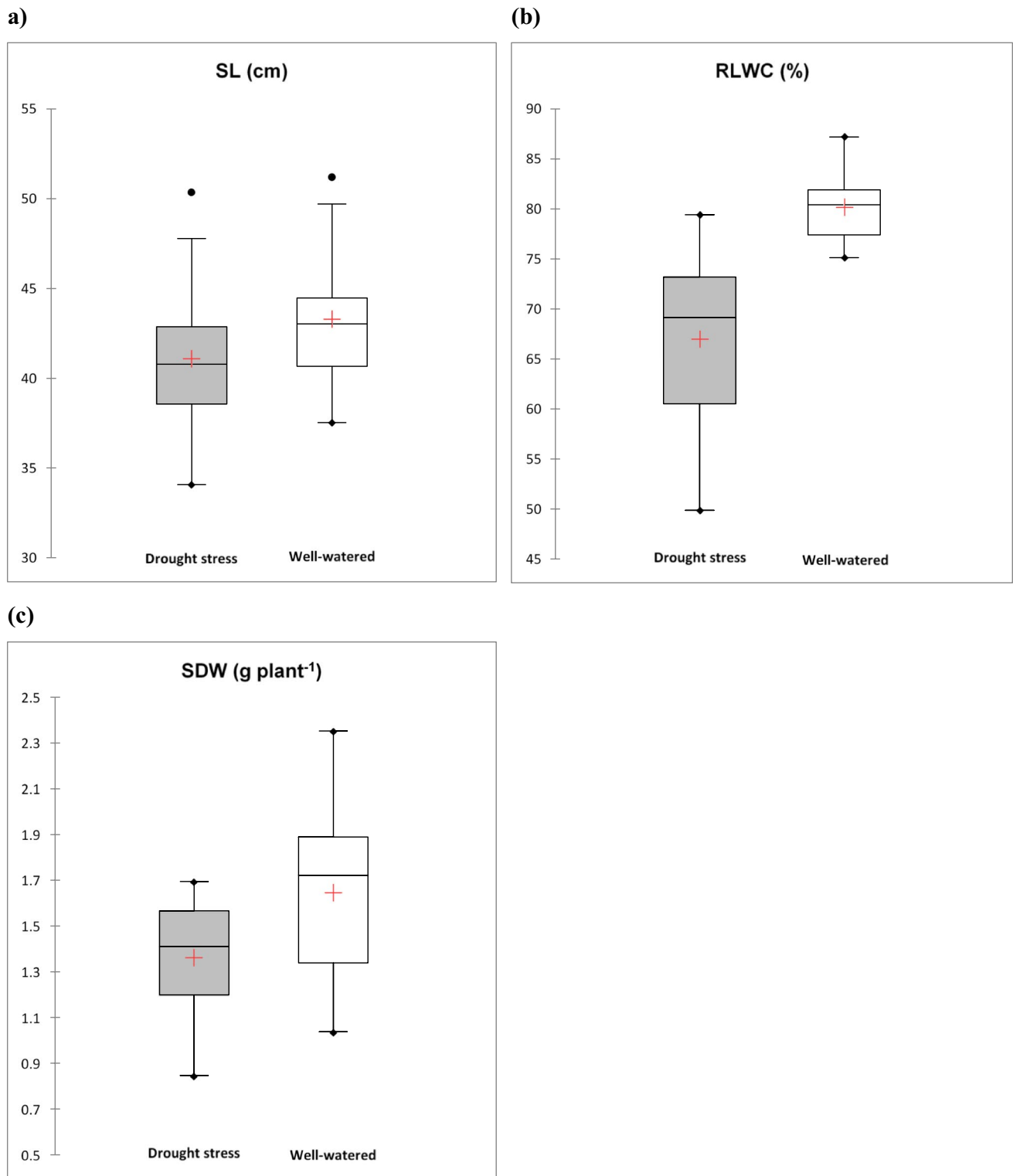


Fig. 1 Box plots showing (a) shoot length (SL), (b) relative leaf water contents (RLWC), and (c) seedling dry weight (SDW) of 20 wheat genotypes under drought stress and well-watered conditions. The box

plots show the median, mean, first quartile, third quartile, minimum, and maximum. Whiskers separate the sample from extreme data point. Data presented beyond whiskers represent outliers

reduced in all genotypes compared to well-watered conditions (Fig. 1).

3.2 Biochemical Attributes

Analysis of variance depicted that drought stress significantly influenced the activities of leaf catalase and superoxide dismutase enzymes (Table 1). The individual effects of drought stress, genotypes, and drought stress \times genotypes were significant ($p \leq 0.05$) for the activities of catalase and superoxide dismutase except for the drought stress \times genotypes for the catalase activity (Table 1). The fitness of the model and R square of the fitness of a model also showed a good sign for activities of leaf catalase and superoxide dismutase (Tables 2S–4S).

Regarding leaf catalase and SOD activities, substantial differences were recorded among different wheat genotypes under both drought stress and well-watered conditions. However, the activities of catalase and SOD enzyme were higher among the tested genotypes under drought stress compared to well-watered conditions (Figs. 1 and 2).

3.3 Physiological Traits

The analysis of variance of different physiological traits (leaf temperature, SPAD chlorophyll contents, intercellular CO_2 concentration, stomatal conductance, net photosynthetic rate, the ratio of CO_2 net assimilation rate and intercellular CO_2 concentration (C_i), transpiration rate, water use efficiency, intrinsic water use efficiency, and instantaneous water use efficiency) showed that drought stress significantly influenced the physiological traits of wheat genotypes (Table 1). The effects of drought stress, genotypes, and drought stress \times genotypes were significant ($p \leq 0.05$) for all the studied physiological traits (Table 1). The fitness of the model and R square of the fitness of a model also showed good significance for different physiological traits (Tables 2S–5S).

Different physiological traits (leaf temperature, SPAD chlorophyll contents, C_i , g_s , A , T , A/C_i , WUE , WUE_i , and WUE_{ins}) showed a substantial genotypic variation under both well-watered and drought stress conditions (Figs. 3, 4, and 5). An overall increase was observed in the leaf temperature, SPAD chlorophyll contents, C_i , WUE , WUE_i , and WUE_{ins} across the genotypes under drought stress compared to well-watered conditions (Figs. 3, 4, and 5). However, an overall decrease in g_s , A , A/C_i , and T was recorded across the genotypes under drought stress compared to well-watered. However, under drought stress, the genotype SQU-95 had a higher net photosynthetic rate than other genotypes compared to well-watered conditions (Fig. 4).

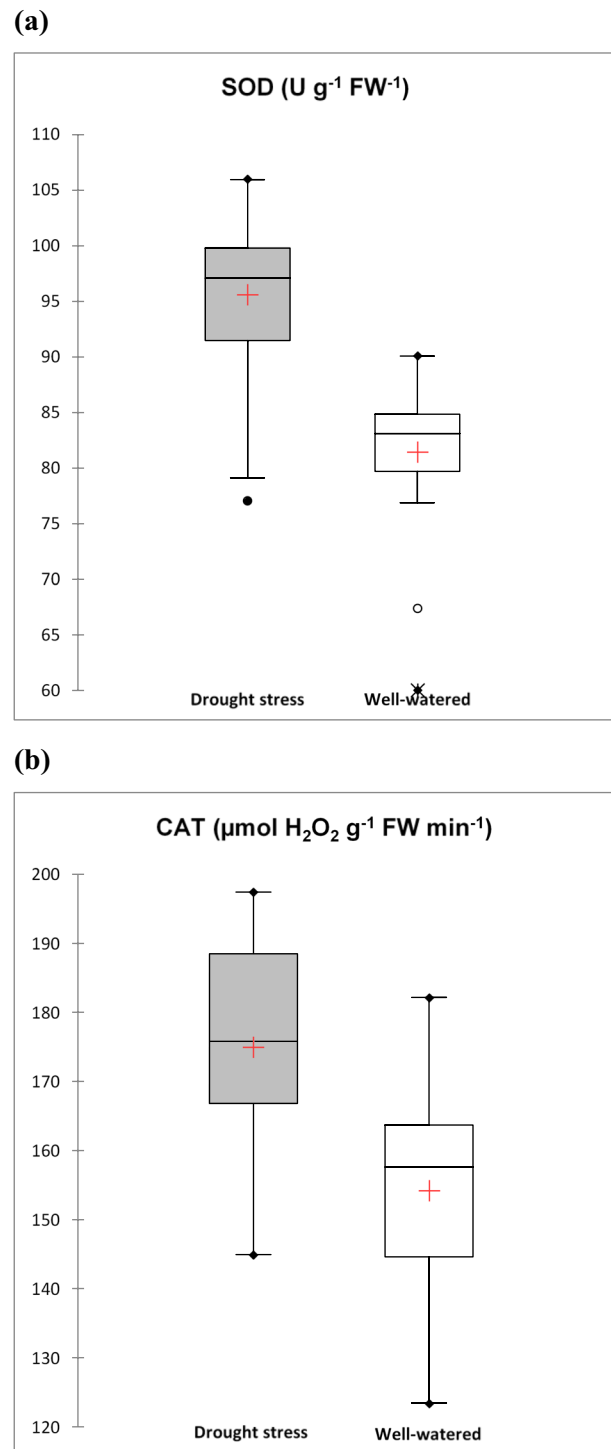


Fig. 2 Box plots showing activities of (a) superoxide dismutase (SOD) and (b) catalase (CAT) of 20 wheat genotypes in response to drought stress and well-watered conditions. The box plots show the median, mean, first quartile, third quartile, minimum, and maximum. Whiskers separate the sample from extreme data point. Data presented beyond whiskers represent outliers

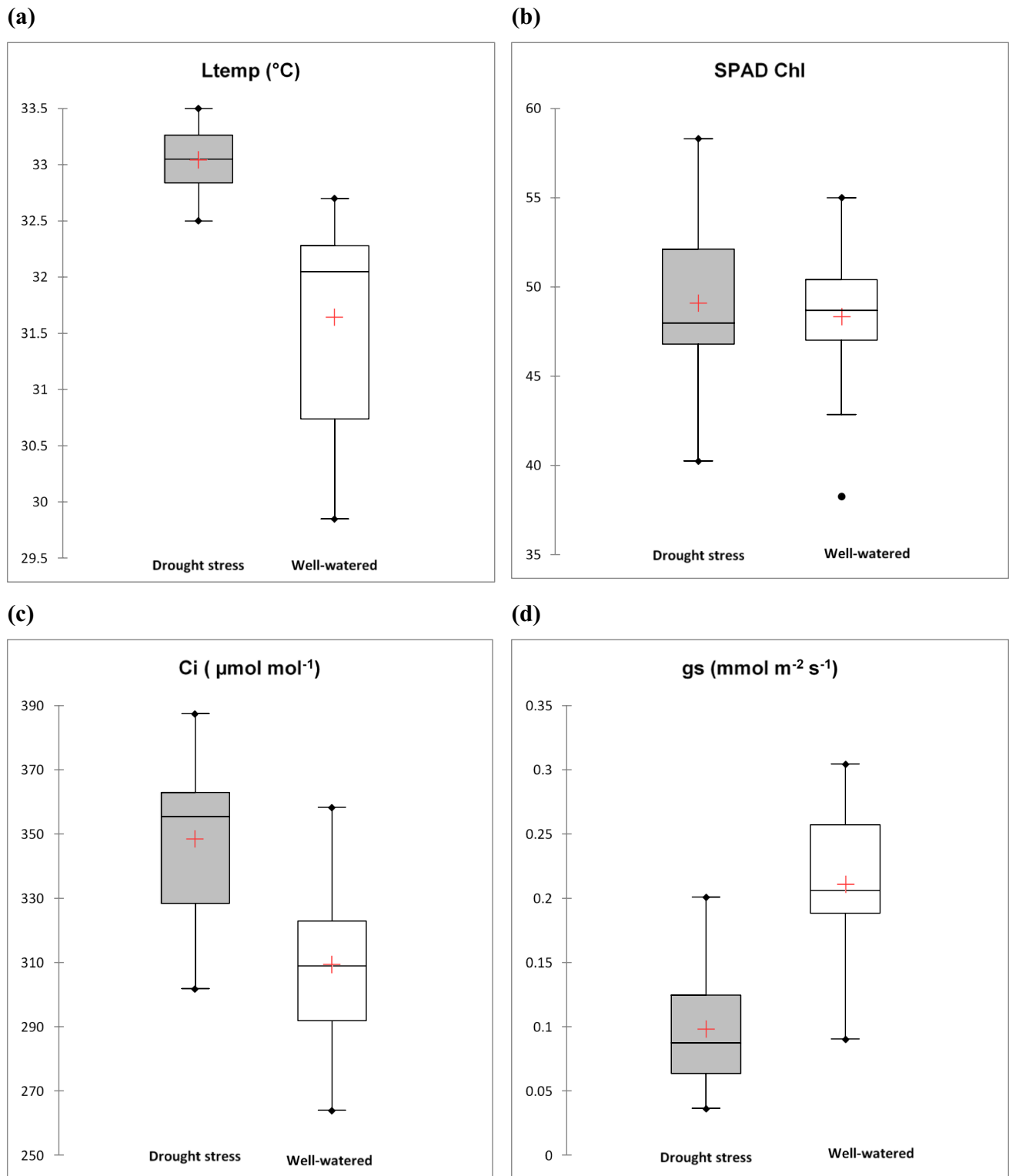


Fig. 3 Box plots showing (a) leaf temperature; (b) SPAD chlorophyll contents; (c) intercellular CO₂ concentration (Ci); and (d) stomatal conductance (gs) of 20 wheat genotypes in response to drought stress and well-watered conditions. The box plots show the median, mean,

first quartile, third quartile, minimum, and maximum. Whiskers separate the sample from extreme data point. Data presented beyond whiskers represent outliers

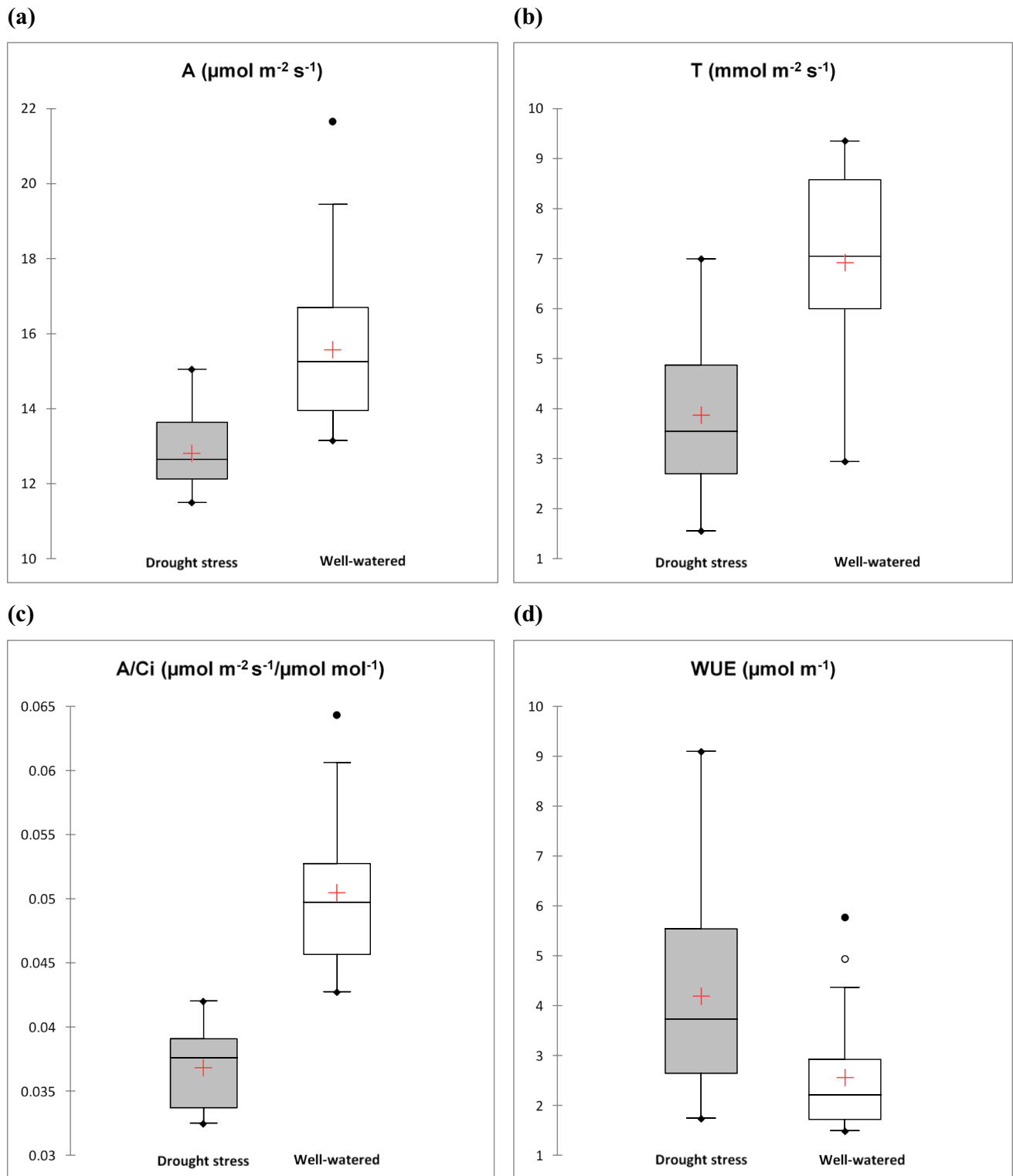


Fig. 4 Box plots showing (a) transpiration rate (T); (b) net photosynthesis (A); (c) ratio of net photosynthesis over intercellular CO_2 concentration (A/Ci); and (d) water use efficiency (WUE) of 20 wheat genotypes in response to drought stress and well-watered conditions.

The box plots show the median, mean, first quartile, third quartile, minimum, and maximum. Whiskers separate the sample from extreme data point. Data presented beyond whiskers represent outliers

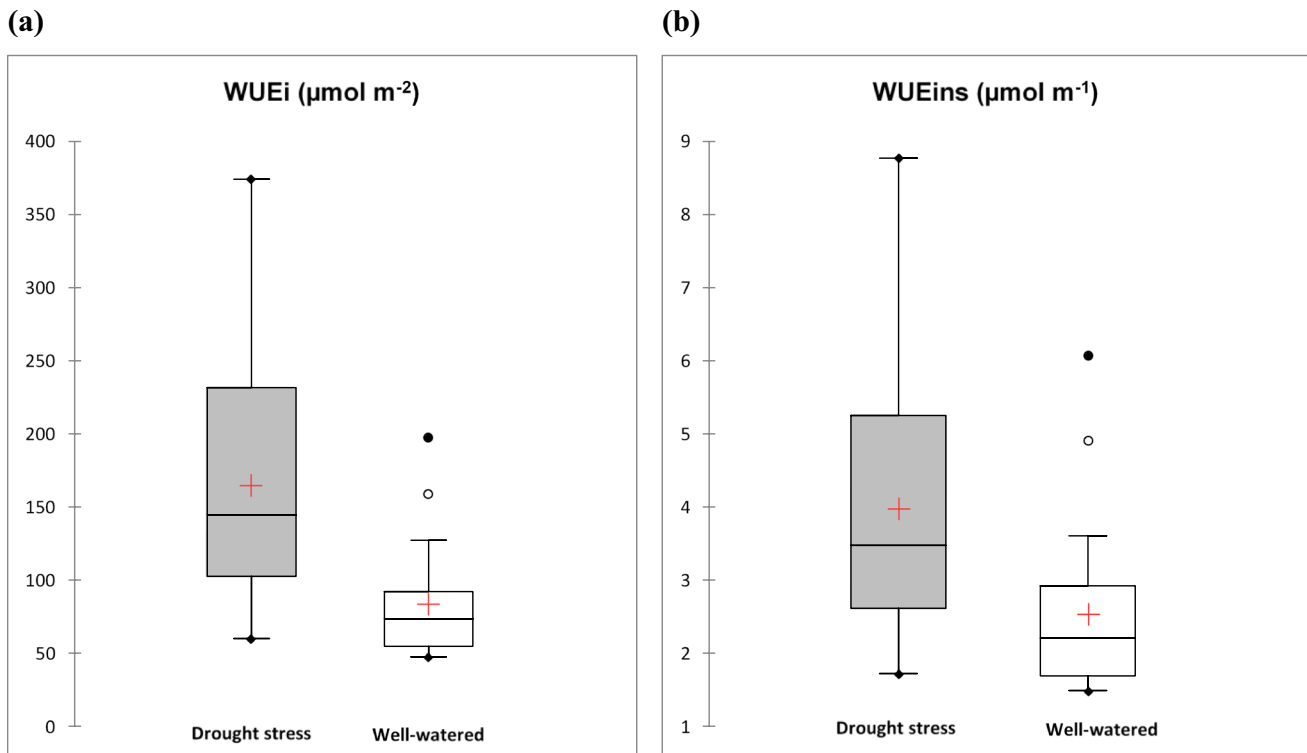


Fig. 5 Box plots showing (a) intrinsic (WUEi) and (b) instantaneous water use efficiency (WUEins) of 20 wheat genotypes in response to drought stress and well-watered conditions. The box plots show the

median, mean, first quartile, third quartile, minimum, and maximum. Whiskers separate the sample from extreme data point. Data presented beyond whiskers represent outliers

3.4 Multivariate Coordination

3.4.1 Morphological and Biochemical Traits

The principal component analysis (PCA) of morphological and biochemical traits under well-watered and drought stress conditions is presented in Fig. 6. The two PCs had 54.0 and 17.0% total variation under well-watered conditions and the leaf shoot length, relative leaf water contents, and seedling dry weight had a negative correlation in PC1, while activities of catalase and superoxide dismutase were positively correlated in PC1 (Fig. 6a). Under drought stress, the two PCs had a significant variation for morpho-biochemical traits and the contribution of variation was 39.1 and 25.6% by PC1 and PC2, respectively. The relative leaf water contents and seedling dry weight were negatively correlated with PC1, while the activities of catalase and SOD were positively correlated with PC1 (Fig. 6b). The genotypes SQU-97, SQU-87, and SQU-88 were grouped based on high catalase and superoxide dismutase activities under drought stress. While genotypes SQU-80 and SQU-82 were grouped due to high leaf shoot length under drought stress (Fig. 6b).

3.4.2 Physiological Traits

The PCA showed significant variation for different studied physiological traits under both well-watered and drought stress conditions. Under well-watered conditions, the two components PC1 and PC2 contributed 65.9 and 14.9% of the total variation, respectively (Fig. 4a). The C_i , A, A/ C_i , WUE, WUEi, and WUEins were positively correlated, while g_s , T, leaf temperature, and SPAD chlorophyll contents were negatively correlated in PC1 (Fig. 7a). The genotypes SQU-79, SQU-80, and SQU-81 were grouped based on high C_i , A, A/ C_i , WUE, WUEi, and WUEins. The genotypes SQU-97, SQU-98, SQU-94, SQU-95, and SQU-90 were grouped based on high g_s , T, leaf temperature, and SPAD chlorophyll value (Fig. 7a).

Under drought, the PC1 contributed 59.2 and PC2 18.4% of the total variability and the C_i , SPAD chlorophyll contents, A, WUE, WUEi, and WUEins were negatively correlated, while g_s , A/ C_i , and T were positively correlated with PC1 (Fig. 7b). The genotypes SQU-97, SQU-95, and SQU-80 were grouped based on high C_i , A, WUE, WUEi, and WUEins, while genotypes SQU-85, SQU-86, SQU-89, and SQU-90 were grouped based on high g_s , T, and A/ C_i (Fig. 7b).

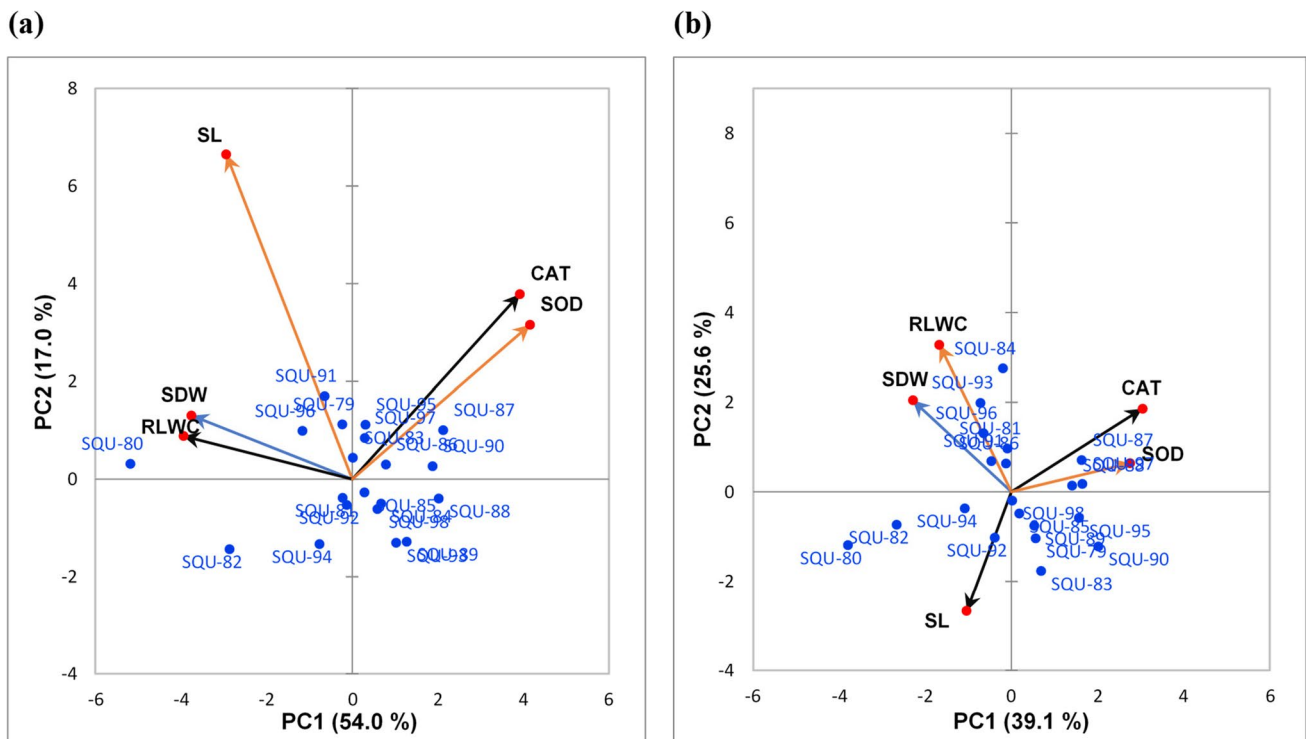


Fig. 6 Principal component analysis of morphological and biochemical traits of 20 wheat genotypes in relation to (a) control and (b) drought stress conditions; SL shoot length; SDW seedling dry

weight; RLWC relative leaf water contents; CAT catalase contents; SOD superoxide dismutase contents

3.4.3 Agglomerative Hierarchical Clustering

The AHC of the wheat genotypes was performed based on studied physiological traits under drought stress on dissimilarity. The tested 20 wheat genotypes were divided into three classes. The variation within the classes was 47.6%, while between the classes was 52.4%. The genotypes SQU-79, SQU-80, SQU-82, SQU-88, SQU-95, and SQU-97 were clustered together into class 1 (Fig. 8). The genotypes SQU-85, SQU-86, SQU-89, and SQU-90 were clustered together into class 3. However, the genotypes SQU-81, SQU-83, SQU-84, SQU-87, SQU-91, SQU-92, SQU-93, SQU-94, SQU-96, and SQU-98 were clustered into class-2 and this class had clustered maximum genotypes compared to class-1 and class-3 (Fig. 8). The class-2 and class-3 had a low variation, while class-1 and class-2 and genotypes SQU-88 and SQU-83 had more variation (Fig. 8).

4 Discussion

The results supported the hypothesis that wheat genotypes with higher intercellular CO_2 concentration and rate of photosynthesis and strong antioxidant defense system can better tolerate drought stress. The genotypes with higher

intercellular CO_2 concentration and rate of photosynthesis maintained better growth under drought than the other genotypes (Fig. 1). Although drought stress caused a reduction in plant biomass, the genotypes SQU-79, SQU-87, SQU-91, SQU-93, and SQU-94 sustained biomass under drought than the other genotypes (Table 6S) because of better carbon assimilation (Table 7S), accumulation of compatible solutes (data not given), and antioxidant potential.

Although a general reduction in plant growth and biomass was observed under drought stress, a few genotypes sustained their growth even under water deficit conditions. In fact, the genotypes capable of osmotic adjustment, due to accumulation of compatible solutes, can minimize the drought-induced decrease in the growth as has been in this study. The sustained growth capability of plants, under drought, is an excellent indicator of drought tolerance and can be employed in selection and breeding programs (Diouf et al. 2018; Sallam et al. 2019). Drought stress also causes oxidative damages to the plants which results in a reduction in photosynthesis and overall plant growth. However, the genotypes better equipped with antioxidant defense system can continue to grow (Ahmadi et al. 2018), although at a lower rate than that of under well-water conditions as has been recorded in this study. Generally, the

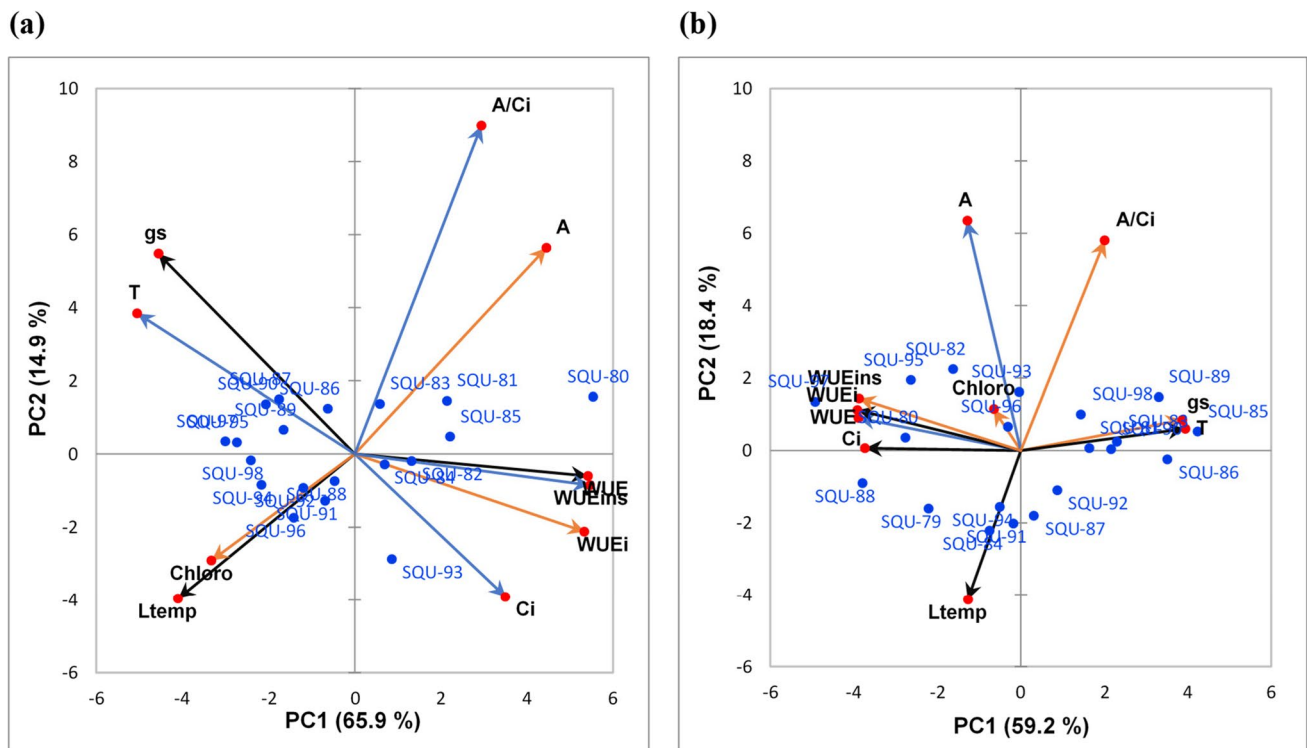


Fig. 7 Principal component analysis of physiological traits of 20 wheat genotypes in relation to **(a)** control and **(b)** drought stress conditions; gs stomatal conductance; Ltemp leaf temperature; T transpiration rate; A photosynthetic rate; Ci intercellular CO₂ concentration;

A/Ci photosynthetic rate/intercellular CO₂ concentration; WUE water use efficiency; WUEi intrinsic water use efficiency; WUEins instantaneous water use efficiency

activities of antioxidant antioxidants increased upon exposure to drought (Figs. 1 and 2); however, only drought tolerant genotypes can sustain this increase during the stress conditions (Guo et al. 2017; Ahmadi et al. 2018). This is important to note that the functioning of this antioxidant defense system and accumulation of compatible solutes is always at the cost of metabolic energy that is otherwise available for plant growth.

Drought stress also significantly affected the gas exchange traits, leaf temperature, SPAD chlorophyll contents, and indices of water use efficiency. A significant decrease was recorded in the gas exchange traits (gs, A, A/Ci, and T) under drought whereas drought caused an increase in leaf temperature, SPAD chlorophyll contents, Ci, WUE, WUEi, and WUEins in the tested genotypes (Figs. 3, 4, and 5). Upon exposure to drought, plants tend to economize the water use through a decrease in the stomatal conductance (Farooq et al. 2009). However, this decrease in stomatal conductance causes a decrease in the influx of CO₂ resulting in a reduction in the net rate of photosynthesis. Therefore, the tolerant please species and genotypes develop a balance between the water loss through transpiration and CO₂ influx required for the carbon assimilation process as has been noted in this study (Table 7S). A very

little decrease in the rate of photosynthesis was recorded in genotypes SQU-82, SQU-89, SQU-93, SQU-94, SQU-95, SQU-96, and SQU-97 under drought stress than other genotypes (Table 7S). However, some non-stomatal factors may also cause a reduction in photosynthesis under drought stress (Pandey and Shukla 2015). These factors involve the stability of the thylakoid membrane, activities of photosynthetic enzymes, triose-phosphate formation, etc. (Farooq et al. 2009). Interestingly, an increase in intercellular CO₂ concentration was noted under drought across the genotypes compared to well-watered conditions (Fig. 3). The increase in Ci, under drought stress, indicates better CO₂ influx despite low stomatal conductance and contributes to improving in WUE and both intrinsic and instantaneous WUE as has been observed in this study (Figs. 4 and 5). The WUE is an important physiological adaptation in plants to improve the productivity of plants under drought stress (Medrano et al. 2015) and after a point of decrease in stomatal conductance, a level exists where a decrease in stomatal conductance increases the WUE in plants (Mashilo et al. 2017). Developing crop genotypes for high WUE is an attractive option to save adequate water for agriculture and food security (Hall and Richards 2013). A higher level of Ci also helps to improve carboxylation efficiency and reducing

References

- Abid M (2016) Improved tolerance to post-anthesis drought stress by pre-drought priming at vegetative stages in drought-tolerant and sensitive wheat cultivars. *Plant Physiol Biochem* 106:218–227. <https://doi.org/10.1016/j.plaphy.2016.05.003>
- Ahmad I, Khaliq I, Khan AS, Farooq M (2014) Screening of spring wheat (*Triticum aestivum* L.) genotypes for drought tolerance on the basis of seedling traits. *Pak J Agric Sci* 51:367–372
- Ahmed HG, Sajjad M, Li M, Azmat MA, Rizwan M, Maqsood RH, Khan SH (2019) Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. *Sustainability* 11:2584. <https://doi.org/10.3390/su11092584>
- Ahmadi J, Pour-Aboughadareh A, Ourang SF, Mehrabi AA, Siddique KHM (2018) Wild relatives of wheat: Aegilops-Triticum accessions disclose differential antioxidative and physiological responses to water stress. *Acta Physiol Plant* 40:90. <https://doi.org/10.1007/s11738-018-2673-0>
- Anjum SA, Tanveer M, Ashraf U, Hussain S, Shahzad B, Khan I, Wang L (2016) Effect of progressive drought stress on growth, leaf gas exchange, and antioxidant production in two maize cultivars. *Environ Sci Pollut Res* 23:17132–17141. <https://doi.org/10.1007/s11356-016-6894-8>
- Anyia A, Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur J Agron* 20:327–339. [https://doi.org/10.1016/S1161-0301\(03\)00038-8](https://doi.org/10.1016/S1161-0301(03)00038-8)
- Bilal M, Rashid RM, Rehman SU, Iqbal F, Ahmed J, Abid MA, Ahmed Z, Hayat A (2015) Evaluation of wheat genotypes for drought tolerance. *J Green Physiol Genet Genom* 1:11–21
- Bowne JB, Erwin TA, Jutner J, Schnurbusch T, Langridge P, Bacic A, Roessner U (2012) Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. *Mol Plant* 5:418–429. <https://doi.org/10.1093/mp/ssr114>
- Barr HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust J Biol Sci* 15:413–428
- Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. *J Exp Bot* 65:1241–1257. <https://doi.org/10.1093/jxb/ert430>
- Crespo-Herrera LA, Crossa J, Huerta-Espino J, Vargas M, Mondal S, Velu G, Payne TS, Braun H, Singh RP (2018) Genetic gains for grain yield in CIMMYT's semi-arid wheat yield trials grown in suboptimal environments. *Crop Sci* 58:1890–1189. <https://doi.org/10.2135/cropsci2018.01.0017>
- Cossani CM, Reynolds MP (2015) Heat stress adaptation in elite lines derived from synthetic hexaploid wheat. *Crop Sci* 55:2719–2735. <https://doi.org/10.2135/cropsci2015.02.0092>
- Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol Plant* 83:463–468. <https://doi.org/10.1111/j.1399-3054.1991.tb00121.x>
- Chance B, Maehly AC (1955) Assay of catalases and peroxidases. *Methods Enzymol* 2:764–775
- Chipilski RR, Kocheva KV, Nenova VR, Georgiev GI (2012) Physiological responses of two wheat cultivars to soil drought. *Z Naturforsch C* 67:181–186. <https://doi.org/10.5560/ZNC.2012.67c0181>
- Diouf IA, Derivot L, Bitton F, Pascual L, Causse M (2018) Water deficit and salinity stress reveal many specific QTL for plant growth and fruit quality traits in tomato. *Front Plant Sci* 9:279. <https://doi.org/10.3389/fpls.2018.00279>
- Dröge W (2002) Free radicals in the physiological control of cell function. *Physiol Rev* 82:47–95. <https://doi.org/10.1152/physrev.00018.2001>
- Farooq M, Gogoi N, Hussain M, Barthakur S, Paul S, Bharadwaj N, Migdadi HM, Alghamdi SS, Siddique KHM (2017) Effects, tolerance mechanisms and management of salt stress in grain legumes. *Plant Physiol Biochem* 118:199–217. <https://doi.org/10.1016/j.plaphy.2017.06.020>
- Farooq M, Hussain M, Siddique KHM (2014) Drought stress in wheat during flowering and grain-filling periods. *Crit Rev Plant Sci* 33:331–349. <https://doi.org/10.1080/07352689.2014.875291>
- Farooq M, Ullah A, Al-Hinai S, Nadaf SK, Al-Sadi AM, Al-Farsi SM, Alkhamisi SA (2022) Wheat production in Oman: experiences, challenges and opportunities. *J Agric Marine Sci* 27:41–49. <https://doi.org/10.24200/jams.vol27iss1pp41-49>
- Farooq M, Ullah A, Lee DJ, Alghamdi SS, Siddique KHM (2018) Desi chickpea genotypes tolerate drought stress better than kabuli types by modulating germination metabolism, trehalose accumulation, and carbon assimilation. *Plant Physiol Biochem* 126:47–54. <https://doi.org/10.1016/j.plaphy.2018.02.020>
- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. *Agron Sustain Dev* 29:185–212. <https://doi.org/10.1051/agro:2008021>
- Farooq MA, Niazi AK, Akhtar J, Saifullah FM, Souri Z, Karimi N, Rengel Z (2019) Acquiring control: the evolution of ROS-induced oxidative stress and redox signaling pathways in plant stress responses. *Plant Physiol Biochem* 141:353–369. <https://doi.org/10.1016/j.plaphy.2019.04.039>
- Fang Y, Liao K, Du H, Xu Y, Song H, Li X, Xiong L (2015) A stress responsive NAC transcription factor SNAC3 confers heat and drought tolerance through modulation of reactive oxygen species in rice. *J Exp Bot* 66:6803–6817. <https://doi.org/10.1093/jxb/erv386>
- Giannopolitis N, Ries SK (1977) Superoxide dismutase. I. Occurrence in Higher Plants *Plant Physiol* 59:309–314. <https://doi.org/10.1104/pp.59.2.309>
- Guo Q, Wang Y, Zhang H, Qu G, Wang T, Sun Q, Liang D (2017) Alleviation of adverse effects of drought stress on wheat seed germination using atmospheric dielectric barrier discharge plasma treatment. *Sci Rep* 7:16680. <https://doi.org/10.1038/s41598-017-16944-8>
- Hall AJ, Richards RA (2013) Prognosis for genetic improvement of yield potential and water-limited yield of major grain crops. *Field Crops Res* 143:18–33. <https://doi.org/10.1016/j.fcr.2012.05.014>
- Han H, Tian Z, Fan Y, Cui Y, Cai J (2015) Water-deficit treatment followed by re-watering stimulates seminal root growth associated with hormone balance and photosynthesis in wheat (*Triticum aestivum* L.) seedlings. *Plant Growth Regul* 77:201–210. <https://doi.org/10.1007/s10725-015-0053-y>
- Huseynova IM (2012) Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. *Biochim Biophys Acta Bioenerg* 1817:1516–1523. <https://doi.org/10.1016/j.bbabi.2012.02.037>
- Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J Exp Bot* 59:3327–3346. <https://doi.org/10.1093/jxb/ern199>
- Joudi M, Ahmadi A, Mohammadi V (2014) Genetic changes in agronomic and phenologic traits of Iranian wheat cultivars grown in different environmental conditions. *Euphytica* 196:237–249. <https://doi.org/10.1007/s10681-013-1027-7>
- Marabottini R, Schraml C, Paolacci AR, Sorgona A, Raschi A, Renzenberg H (2001) Foliar antioxidant status of adult Mediterranean oak species (*Quercus ilex* L. and *Q. pubescens* Willd.) exposed to permanent CO₂ enrichment and to seasonal water stress. *Environ Pollut* 115:413–423. [https://doi.org/10.1016/s0269-7491\(01\)00231-7](https://doi.org/10.1016/s0269-7491(01)00231-7)

- Martin B, Ruiz-Torres NA (1992) Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticumaestivum* L.). *Plant Physiol* 100:733–739. <https://doi.org/10.1104/pp.100.2.733>
- Mashilo J, Odindo AO, Shimelis HA, Musenge P, Tesfay SZ, Magwaza LS (2017) Drought tolerance of selected bottle gourd [*Lagenariasiceraria* (Molina) Standl.] landraces assessed by leaf gas exchange and photosynthetic efficiency. *Plant Physiol Biochem* 120:75–87. <https://doi.org/10.1016/j.plaphy.2017.09.022>
- Medrano H, Tomasa M, Martorell S, Flexas J, Hernandez E, Rossello J, Poub A, Escalona JS, Bota J (2015) From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. *Crop J* 3:220–228. <https://doi.org/10.1016/j.plaphy.2017.09.022>
- Mwadzingeni L, Shimelis H, Dube E, Laing MD, Tsilo TJ (2016) Breeding wheat for drought tolerance: progress and technologies. *J Integr Agric* 15:935–943. [https://doi.org/10.1016/S2095-3119\(15\)61102-9](https://doi.org/10.1016/S2095-3119(15)61102-9)
- Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol* 49:249–279. <https://doi.org/10.1146/annurev.arplant.49.1.249>
- Pandey V, Shukla A (2015) Acclimation and tolerance strategies of rice under drought stress. *Rice Sci* 22:147–161. <https://doi.org/10.1016/j.rsci.2015.04.001>
- Reynolds MP, Pask AJD, Hoppitt WJE, Sonder K, Sukumaran S, Molero G, Saint Pierre C, Payne T, Singh RP, Braun HJ (2017) Strategic crossing of biomass and harvest index – source and sink — achieves genetic gains in wheat. *Euphytica* 213:257. <https://doi.org/10.1007/s10681-017-2040-z>
- Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol* 161:1189–1202. <https://doi.org/10.1016/j.jp1ph.2004.01.013>
- Sallam A, Alqudah AM, Dawood MF, Baenziger PS, Börner A (2019) Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. *Int J Mol Sci* 20:3137. <https://doi.org/10.3390/ijms20133137>
- Schwanz P, Polle A (2001) Differential stress responses of antioxidative systems to drought in pendunculate oak (*Quercusrobur*) and maritime pine (*Pinuspinaster*) grown under high CO₂ concentrations. *J Exp Bot* 52:133–143. <https://doi.org/10.1093/jexbot/52.354.133>
- Stiling P, Moon D, Rossi A, Forkner R, Hungate BA, Day FP, Drake B (2013) Direct and legacy effects of long-term elevated CO₂ on fine root growth and plant-insect interactions. *New Phytol* 200:788–795. <https://doi.org/10.1111/nph.12295>
- Trnka M, Feng S, Semenov MA, Olesen JE, Kersebaum KC, Rötter RP, Semerádová D, Klem K, Huang W, Ruiz-Ramos M, Hlavinka P, Meitner J, Balek J, Havlík P, Buntgen U (2019) Mitigation efforts will not fully alleviate the increase in water scarcity occurrence probability in wheat-producing areas. *Sci Adv*. <https://doi.org/10.1126/sciadv.aau2406>
- Ullah A, Farooq M (2021) The challenge of drought stress for grain legumes and options for improvement. *Arch Agron Soil Sci* 29:1–8. <https://doi.org/10.1080/03650340.2021.1906413>
- Zahra N, Wahid A, Hafeez MB, Ullah A, Siddique KHM, Farooq M (2021) Grain development in wheat under combined heat and drought stress: plant responses and management. *Environ Exp Bot* 188:104517. <https://doi.org/10.1016/j.envexpbot.2021.104517>
- Zhang S, Dang QL (2005) Effects of soil temperature and elevated atmospheric CO₂ concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. *Tree Physiol* 25:523–531. <https://doi.org/10.1093/treephys/25.5.523>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.