

Analysis of Physio-biochemical responses and expressional profiling of DREB transcription factors for drought stress tolerance in *Aegilops tauschii* Coss

Adeel Abbas

Jiangsu University

Rashida Hameed

Jiangsu University

Aitezaz Shahani

Yanbian University

Wajid Ali

Jiangsu University

Ping Huang (✉ huangjiehp@ujs.edu.cn)

Jiangsu University <https://orcid.org/0000-0002-1988-3638>

Yizhou Du

The University of Sydney

Daolin Du

Jiangsu University

Research Article

Keywords: Drought tolerance, abiotic stresses, photosynthesis activity, antioxidant system. Gene expression analysis

Posted Date: April 11th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2775394/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on July 18th, 2023. See the published version at <https://doi.org/10.1007/s10722-023-01661-1>.

Abstract

Aegilops tauschii Coss. ($2n = 2x = 14$, DD) is a weed of wheat, competes for resources, performs better against different abiotic stresses, and is a rich genetic material source for wheat crop improvement programs. The effects of different drought stress levels on physiological and biochemical parameters and the expression profile of the drought resistance gene DRB were examined through greenhouse experiments. The physiological parameters (plant height, dry weight biomass, plant height, and leaf area-related parameters) fluctuated under different drought stress levels. Photosynthetic parameters (maximum (F_o') and minimum ($F'm$) fluorescence, photochemical ($(\Phi PSII)$), and non-photochemical quenching (qN)) were also affected by different drought stress levels, and their efficiency fluctuated with water availability. In the biochemical analysis, shallow significant changes occurred in peroxidase (POD), catalase (CAT), and proline content, while malondialdehyde (MDA) showed variation under different drought stress conditions. In the *DREB* gene expression analysis, eight populations showed high expression profiles compared to the control conditions in *Ae. tauschii*. The expression profiles in *Ae. tauschii* showed a high fold increase compared to the control condition, and *DREB* gene expression correlated with the changes in physiological and biochemical parameters.

1. Introduction

Extended drought stress periods, heat stress, and heavy climate change are becoming more regularly affecting global crop production [1]. Drought stress is the most distressing threat to agricultural productivity worldwide [2]. It has been assessed that by 2050, worldwide drought will adversely affect plant growth and development and ultimately decrease productivity [3]. Drought stress reduces photosynthesis, relative water content, cell division and elongation and causes an imbalance between antioxidant and reactive oxygen species [4]. Under different abiotic stresses, including drought, plants change their physiological, biochemical, and molecular processes [5]. Different drought stress conditions directly influence different plant growth parameters, chlorophyll content, and photosynthetic activities [2]. Water stress damages cell inhibition, growth, induction of the repair system, and different metabolic activities in plants [6].

Plant responses to different drought conditions are fundamental for implementing crop management strategies to stimulate physiological, biochemical, and molecular changes [7], disturbing the physiological process of reactive oxygen species (ROS) levels in plant cells [8]. Disproportionate and excessive accumulation of ROS, exemplified by superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\cdot$), and singlet oxygen (O_2), leads to the generation of oxidative stress [9]. During the management of drought stress with oxidative damage, plants change the levels and/or activity of several enzymes, including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in plants (Satti et al. 2022) to avoid or repair the damage caused by the production of ROS [10], in plants [11]. SOD, Ascorbate peroxidase (APX), POD, and CAT activities are vital in protecting plants from severe drought stress [12]. These enzymes make plants tolerant to different extreme biotic and abiotic stresses [13, 14].

Antioxidant enzymes (POD, SOD, and CAT) have been studied in different varieties of lantana (pink, red, and violet) under different environmental conditions [15, 16]. Several genes are involved in drought stress tolerance, linked to different plant morphological and structural changes [17]. Drought tolerance has complex phenotypic and quantitative traits that include these responses and involve several different genes [18].

Different candidate and responsive genes in cereals control adaptive strategies against various abiotic stresses, including drought [19]. Transcription factors (*TFs*) play vital roles in plant growth and development under different biotic and abiotic stresses [20]. Among *TFs*, the dehydration-responsive element binding protein (*DREBs*) family plays essential roles in plant signalling networks and modulates many methods [21]. *DREB* expression has been investigated under different abiotic stresses in different plant species [22]. However, recent studies have shown inconsistencies in the expression of *DREB* under different abiotic stresses, including drought and salinity stress conditions [23]. Some studies have reported that *DREB* affects and responds to adverse salinity, drought, and low-temperature environments [24]. To understand the regulatory mechanisms and molecular networks activated under different drought stress levels is very important to identify genes mediating tolerance to these conditions in various plant species [25].

Ae. tauschii is a rough and wild species, and its D genome is considered to be a donor for cultivated wheat [26, 27]. It has broad geographical distribution in different wheat-growing countries, including China, Turkey, Central Asia, and Afghanistan. In China, its large-scale effects compete for different sources in wheat production areas [28, 29]. It has the ability to perform better against different abiotic stresses (heat, drought, and salinity) and has quick adaptation strategies [30, 31]. D genome of *Ae. tauschii* is a rich genetic source, and its useful traits (resistance to drought, salinity, and heat) can be used for wheat development programs [28, 32]. In this study, we evaluated different physiological, biochemical, and molecular parameters that are directly linked to drought stress and provided evidence for understanding the drought stress mechanism in *Ae. tauschii* Coss.

2. Material And Method

2.1 Plant Materials

Ae. tauschii seeded were collected from 5 provinces of China (Shandong, Shanxi, Shaanxi, Henan, and Hebei). Greenhouse experiments were conducted at the institute of plant protection Chinese Academy of Agriculture Sciences in Beijing, China. A complete randomized design was performed with eight replications, and data were recorded for various physiological and biochemical parameters. For drought stress application, three different levels of field capacity $D_1 = 15\text{--}25\%$, $D_2 = 45\text{--}55\%$, and $D_3 = 75\text{--}85\%$ were applied, and Time-Domain Reflectometry (TDR) soil moisture meter (TRIMEPICO, Germany) was used to measure the moisture level. Before 15 days of drought stress application, 80–100% moisture levels were maintained in pots (size 13cm in height and 15cm in diameter) for seed germination. The pot contained sandy loam soil and hummus at a ratio of 3:1.

2.2 Physiological parameters and Chlorophyll fluorescence

Physiological parameters (plant height, dry weight biomass, leaf area, and specific leaf area) were measured under different drought stress levels. Meter rod and portable leaf area meters were used to estimate plant height and leaf area. Plants were separated into root, shoot for dry biomass, and placed in an oven for 48 hours. Digital weight balance was used to calculate dry biomass—the dry weight ratio to leaf area measured by specific leaf area (SLA). For chlorophyll and photosynthesis parameters, imaging-PAM (IMAG-K5, walz, Germany) was used to calculate maximum (F_o') and minimum ($F'm$) fluorescence, photochemical ($(\Phi PSII)$) and non-photochemical quenching (qN) [33].

2.3 Determination of biochemical indices

Measurements of antioxidant defence systems include the content of POD, CAT, proline, and MDA [34]. POD activity was determined by the guaiacol method; The activity of CAT was determined by the UV absorption method [35]; Determination of proline by acidic ninhydrin of MDA content by thiobarbituric acid method [33].

2.4 RNA Extraction and cDNA Synthesis

For gene expression analysis, 8 populations were used under control and drought (75 g L^{-1} PEG-6000) stress with 3 replications for each population. After 15 days of drought stress application, leaf sample of each population was harvested and stored at -80°C . Ribonucleic acid (RNA) preparation pure plant kit (Cat. #DP432 Tiangen Biotechnology Beijing CO., LTD China) was used for RNA extraction. From 1g of leaf sample, genomic DNA was extracted by using RNase-free DNase1 kit (Taingen, Biotechnology CO., LTD Beijing, China). The concentration and integrity of DNA was checked at 1% agarose gel electrophoresis, and RNA quantity and quality was measured at NanoDrop (NanoDrop Technologies, Wilmington, DE, USA). The $1\mu\text{g}$ of total RNA was used for cDNA synthesis using fast quant R.T. kit (Cat #KR116-02, Tiangen Biotechnology CO., LTD Beijing, China), and the sample was stored at -80°C for further usage.

2.5 Primer and qPCR analysis

For gene dehydration-responsive element-binding protein 1E (*DREB*) expression studies, the primers; *DREB-F*: CGAGTCTGTTGATGAGTCT and *DREB-R* TTCTGTAGTAAGTGCTTGCTA were used, respectively. The actin was used as reference gene for qPCR analysis *ACT-F* ACT-F TTGCCTTGGATTATGAACA and *ACT-R* GATGGCTGGAACAGAACTT were used respectively. For qPCR, ABI 7500 machine was used by applying SYBR green (Applied Biosystems, Foster City, CA, USA). Total volume of 1 sample was $20\mu\text{L}$, which contained $10\mu\text{L}$ PCR master mix, $0.6\mu\text{L}$ of forward and reverse primer, $0.6\mu\text{L}$ of dye and $7.3\mu\text{L}$ of ddH_2O . Cycling condition for melting curve temperature: 10 minutes at 95°C , $55-58^\circ\text{C}$ for 32 seconds, 40 cycles for 95°C , with an increase of 0.5 to 0.7°C every 5 seconds and extended at 72°C for 30 seconds. Four biological and three technical replicates were applied during qPCE analysis.

3. Results

3.1 Physiological and chlorophyll parameters

Physiological and chlorophyll parameters were significantly affected by drought stress. The dry-weight biomass allocation of *Ae. tauschii* under different drought stress levels was significantly ($p<0.05$) affected, and minimum and maximum dry weight biomass (0.65 g and 1.65 g) were recorded under 75% and 15-25% field capacity levels, respectively. The maximum decreased by 55% in dry weight biomass under 15-25% drought stress levels. Under different drought levels, the maximum plant height was 16.4 cm at 75-85%, while the minimum was 11.3 cm under 15-25% drought stress application. Plant height was reduced by 21% under 15-25% drought stress. Regarding root shoot biomass allocation, 0.64 g were recorded in high field capacity level, in contrast, a minimum of 0.54 g was recorded in the lower field capacity level. The reduction percentage was 13.4% in lower field capacity levels as compared with high field capacity. For leaf area synthesis, drought stress decreased production; a maximum of 114.54 cm² was recorded in 75-85% field capacity, whereas a minimum of 64.43% was recorded in lower capacity levels. A 38% decrease occurred compared with lower water availability. Leaf area ratio (LAR) and specific leaf area (SLA) significantly affected the maximum (154.54 and 422.43%, respectively) measured under high water availability, while the minimum was (39.11 and 75.43) at lower field capacity levels (10.20%). Reduction in LAR and SLA was 67% and 73%, respectively, at lower capacity levels of 15-25%.

Chlorophyll fluorescence was also significantly affected by drought stress. F_o' and F_m' steadily decreased with increasing water availability. F_m' measured a maximum of 0.48 in high field capacity and a minimum of 0.32 in lower field capacity. In the quantum yield of photosystem II (PSII), the maximum and minimum (0.51 and 0.34) were recorded under high (75-85%) and lower (15-25%) levels. A similar trend was observed for photosystem II. Photochemical quenching also increased to 0.54 at high water availability, reaching a minimum of 0.43 at the lower field capacity level. In q_N , it was 0.65, while it decreased and reached 0.54 at 15-25% field capacity levels.

Table 1. Minimum, maximum and mean values of physiological traits ((plant height, dry weight biomass, leaf area, leaf area ratio and root and shoot ratio) under different drought stress levels in *Ae. tauschii*.

	Plant Height (cm)	Dry Weight of Biomass	Leaf area	Specific leaf area	Leaf area ratio	R/S Ratio
D1						
Mini.	13.5	1.87	77.4	54.9	31.41	0.63
Maxi.	15.7	2.78	89.5	150.6	67.09	0.87
Mean	15.1	2.35	82.4	15.1	49.33	0.72
D2						
Mini.	11.5	1.56	72.4	129.0	70.90	0.64
Maxi.	13.7	2.11	83.4	170.6	94.85	0.98
Mean	13.1	1.92	78.4	13.1	82.29	0.78
D3						
Mini.	11.5	1.65	69.5	159.8	77.98	0.54
Maxi.	14.5	2.31	79.4	324.7	81.42	0.94
Mean	12.7	1.92	72.9	12.7	108.18	0.76

D₁= 15-25%, D₂= 45-55% , D₃= 75-85%, Mini=minimum and maxi=maximum.

Table 2. Effect of different drought stress level on the physiological traits (plant height, dry weight biomass, leaf area, leaf area ratio and root and shoot ratio) in *Ae. tauschii*.

Treatments	Plant Height (cm)	Dry Weight of Biomass	Leaf area	Specific leaf area	Leaf area ratio	R/S Ratio
D1D2	1.96*	0.42*	4.75*	-59.65*	-33.61*	-0.06
D1D3	2.01*	0.36*	9.07*	-65.47*	-64.66*	-0.031
D2D1	-1.96*	-0.42*	-4.75*	59.65*	33.61*	0.06
D2D3	0.05	-0.05	4.32*	-5.82	-31.05*	0.028
D3D1	-2.01*	-0.36*	-9.07*	65.47*	64.66*	0.032
D3D2	-0.05	0.053	-4.32*	5.825	31.052*	-0.028

The different letter shows a significant difference in treatments at 5% probability level. D₁= 15-25%, D₂= 45-55% and D₃= 75-85%

Table 3. Minimum, maximum and Mean values of physiological traits under control and salinity stress conditions.

	Fo'	F'm	Y(II)	ΦPSII	qN	qP	NPQ	Y(NPQ)
D1								
Mini.	0.21	0.34	0.37	0.28	0.54	0.43	0.34	0.24
Maxi.	0.34	0.53	0.54	0.63	0.75	0.76	0.48	0.49
Mean.	0.28	0.42	0.45	0.46	0.68	0.56	0.39	0.36
D2								
Mini.	0.11	0.34	0.31	0.24	0.54	0.32	0.28	0.29
Maxi.	0.31	0.48	0.45	0.45	0.65	0.64	0.45	0.45
Mean.	0.22	0.39	0.38	0.38	0.61	0.46	0.37	0.37
D3								
Mini.	0.14	0.28	0.25	0.21	0.45	0.34	0.26	0.24
Maxi.	0.26	0.39	0.41	0.43	0.74	0.55	0.43	0.41
Mean.	0.19	0.33	0.33	0.31	0.55	0.43	0.37	0.31

(Fo'=minimal fluorescence, F'm= maximal fluorescence, Y(II)= effective quantum yield of PSI ΦPSII= quantum yield of PSII photochemical, qP= photochemical quenching, qN =non-photochemical quenching, NPQ= Nonphotochemical chlorophyll fluorescence quenching and Y(NPQ)= fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism).

Table 4. Effect of different drought stress level on different photosynthetic parameters under different drought stress levels.

Treatments	Fo'	F'm	Y(II)	ΦPSII	qN	qP	NPQ	Y(NPQ)
D1D2	0.058*	0.034	0.070*	-0.095	0.075	0.092	0.017	-0.009
D1D3	0.085*	0.098*	0.122*	0.045	0.13*	0.125*	0.03	0.045
D2D1	-0.058*	-0.04	-0.071*	0.008	-0.075	-0.092	-0.017	0.009
D2D3	0.03	0.065*	0.052*	0.053	0.055	0.032	0.009	0.061
D3D1	-0.086*	-0.09*	-0.13*	-0.045	-0.13*	-0.12*	-0.025	-0.05
D3D2	-0.03	-0.066*	-0.052*	-0.053	-0.06	-0.03	-0.008	-0.054

The different letter shows a significant difference in treatments at 5% probability level. D₁= 15-25%, D₂= 45-55% and D₃= 75-85%. Fo'=minimal fluorescence, F'm= maximal fluorescence, Y(II)= effective quantum yield of PSI ΦPSII= quantum yield of PSII photochemical, qP= photochemical quenching, qN =non-photochemical quenching, NPQ= Nonphotochemical chlorophyll fluorescence quenching and Y(NPQ)= fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism).

3.2 Drought stress and antioxidant system

When plants suffer from drought stress, their bodies' cellular oxidation balance is destroyed. Our study found that POD and CAT responded actively to oxidative damage in *Ae. tauschii* plants under different drought stress levels. Drought stress treatment had a slight effect on POD activity and no significant effect on leaf POD content. However, the CAT content decreased significantly with increasing drought stress treatment in *Ae. tauschii*. As one of the important components of plant proteins, proline is free in plants, is produced in large quantities, and accumulates in plants under abiotic stresses, such as high salt and drought. Our results showed that the levels of proline decreased in *Ae. tauschii* with increasing drought stress. In contrast, MDA content decreased significantly with under drought conditions in *Ae. tauschii*. Its content decreased dramatically and reached a very low level compared to the control with high water availability.

Table 5. Minimum, maximum, and Mean values of antioxidant system under different drought stress level.

	POD	CAT	MAD	Proline
D1				
Mini.	1209	323	3.11	3.11
Maxi.	1420	454	3.98	3.98
Mean	1351.9	383.9	3.56	3.76
D2				
Mini.	1098	311	2.54	2.54
Maxi.	1320	367	3.34	3.21
Mean	1237.3	344.5	2.99	2.99
D3				
Mini.	987	231	2	2
Maxi.	1202	363	2.94	2.94
Mean	1107.8	272.0	2.33	2.33

D₁= 15-25%, D₂= 45-55% and D₃= 75-85%. CAT= catalase, POD= peroxidase, MAD= malondialdehyde).

Table 6. Effect of different drought stress level on antioxidant system in *Ae. tauschii*

Treatments	CAT	POD	MAD	Proline
D1D2	39.37	114.63*	0.543*	0.59
D1D3	111.87*	244.13*	1.052*	1.355*
D2D1	-39.37	-114.63*	-0.543*	-0.59
D2D3	72.50*	129.51*	0.508*	0.756*
D3D1	-111.87*	-244.13*	-1.052*	-1.35*
D3D2	-72.50*	-129.51*	-0.508*	-0.756*

The different letter shows a significant difference in treatments at 5% probability level. D₁= 15-25%, D₂= 45-55% and D₃= 75-85%. CAT= catalase, POD= peroxidase, MAD= malondialdehyde).

3.3 Expression Patterns of DRB under drought stress

Expression analysis of *DRB* under control and drought stress levels (75 g L⁻¹ PEG-6000). Eight populations showed substantial changes under different drought stress conditions. Beacon Designer software designed forward and reverse primer (5'-3') of reference genes and used within in exon regions. Exon-exon region primers are specific for the amplification of cDNA, which contains an intron between exon-exon junctions. The expression profile of the *DRB* gene varies, and high expression has been reported under drought-stress conditions. Under drought stress (75 g L⁻¹ PEG-6000), expression levels of *DRB* were higher than those under normal conditions. Under control conditions, the *DRB* expression was 1.2-, 1.3-, 1.5-, 1.3-, 1.6-, 1.3-, 1.4-, and 1.2-fold higher than that under normal conditions. When drought stress treatment was applied, the expression of *DRB* was 5.3-, 5.4-, 4.6-, 5.1-, 4.9-, 5.8-, 5.9-, and 5.3-fold higher than that under control conditions.

4. Discussion

In our study, we investigated physiological, biochemical, and molecular parameters in *Ae. tauschii* populations under different drought stress conditions. *Ae. tauschii* present in more than 10 provinces of China and infests 330,000 hectares of wheat-growing area [36, 37]. In this study, we investigated the mechanisms of *Ae. tauschii* to drought stress environments to understand its adaptability and performance. *Ae. tauschii* can utilize more water and soil resources and has higher growth rates than other crops [38]. At the initial experiments regarding physiological parameters, drought stress was found to affect leaf area, biomass allocation and root and shoot development. Water availability is vital for plant growth and affects cellular processes [39]. *Ae. tauschii* significantly increased root and shoot growth in water shortage and survived better in harsh environments, even though water availability

directly affects growth and biomass allocation [40]. Therefore, the *Ae. tauschii* genome has a natural ability to withstand different environmental stresses [41]. Similarly, *Prunus mongolica* affected the root-to-shoot ratio under different drought stress levels [42]. *Plantago ovata* and *psyllium* also significantly affected physiological parameters under different drought stress conditions [43]. Drought stress conditions did not suppress plant height and leaf area because they can grow faster than normal plants [28, 44].

Chlorophyll fluorescence is an important method for determining the photosynthetic activity of plant leaves under different abiotic stresses [36, 45]. Plant leaves are sensitive to different abiotic stress conditions and fluctuate their photosynthetic rates through photosystems [46]. In the current study, different drought stress levels significantly decreased chlorophyll fluorescence. Under different drought stress levels, F_o increased significantly, while F_m decreased with different drought stress levels. F_o is a powerful component under different abiotic stresses, and our study showed that drought stress levels exchange light energy by inhibiting the potential active centre [47]. qP emulates and increases plant F_o and decreases F_m or the F_v/F_m ratio.

Under different drought stress conditions, qP and qN decreased in *Ae. tauschii*. PS II is an essential component of qP, and qN secures the plant protection system under different abiotic stresses, including drought [48]. Drought stress significantly decreases and damages PS II and reduces the thrashing of heat extravagance potential of plants [49]. Our results displayed that the long-term unavailability of water may affect the photosynthesis process in *Ae. tauschii*, which partially or completely suppressed the qP activity. Moreover, drought and other abiotic stresses also affect large-scale chlorophyll fluorescence in *Beta vulgaris*, *Coronilla varia*, *P. mongolica* and *L. ruthenicum* [50, 51]. Drought stress can destroy plant cell membrane systems [52], and many studies have shown that CAT, POD, MDA, and free proline accumulate in plants under drought stress to maintain the dynamic balance of the antioxidant system [53, 54]. To avoid or alleviate cell damage caused by ROS, plants stimulate their antioxidant enzyme systems, which are closely related to plant stress resistance [55]. CAT can prevent cell membrane damage, POD can remove excessive reactive oxygen species, MDA is the final product of lipid peroxidation in plants, and the accumulation capacity of free proline represents the drought resistance of plants [54, 56–58]. It combines and cross-links with proteins on the cell membrane to destroy the structure and function of the biofilm, and its content can represent the degree of cell membrane damage [59].

The results of the present study show that *Ae. tauschii* significantly increased CAT, proline, and MDA contents in their leaves under drought conditions, and no significant changes were observed for POD content. The content of CAT, proline, and MDA in *Ae. tauschii* leaves were positively correlated with drought severity during natural drought. These results suggested that, *Ae. tauschii* reduced its oxidative damage caused by the increased concentration of free proline content and regulated the activity of antioxidant enzymes under various drought stress levels. After physiological and biochemical analysis, we exposed eight populations of *Ae. tauschii* for drought resistance gene analysis. The *DREB* gene can be considered an important regulator in drought stress tolerance network in many plants, including wheat

and *Arabidopsis* [13, 46] and helpful in the adaptation of wheat under various drought stress environments [60]. On the basis of some previous findings on *Ae. tauschii* [28, 30, 36], and in current study, we used 8 different populations for gene expression analysis for various physiological and biochemical attributes. We selected the *DREB* gene to explore the expression profile under drought stress conditions [61]. *DREBs* a, transcription factor modulates different abiotic stress, including drought stress [62]. In different plant species, *DREBs* expression profiles were investigated at an extensive rate [63]. However, few plant species displayed variation in their expression profile [23], and it's linked with chlorophyll, proline content, and sugar content of different plants [64]. In the current study, the expression profile in *Ae. tauschii* showed a high fold compared to the control condition, which shows changes in their link with different physiological and biochemical attributes [65]. Our experiments showed that higher expression of *DREBs* resulted owing to complex process of gene transcription. This *DREB* gene can act as a point of interaction or involve several pathways to regulate drought stress condition and can be a valuable resource to utilize in future studies as well.

Declarations

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (32271587 and 31200316), Carbon peak and carbon neutrality technology innovation foundation Jiangsu province (BK20220030), the Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Study Abroad Scholarship of Jiangsu University, and Senior Talent Fund of Jiangsu University (11JDG150). The authors are thankful to ediatge (<https://www.editage.com/>) for providing with quality scientific language editing services.

References

1. Zhu X et al (2022) Manure amendment can reduce rice yield loss under extreme temperatures. *Commun Earth Environ* 3:1–9
2. Wang Z et al (2022) ZmERF21 directly regulates hormone signaling and stress-responsive gene expression to influence drought tolerance in maize seedlings. *Plant Cell Environ* 45:312–328
3. Roy P et al (2022) Climate change and groundwater overdraft impacts on agricultural drought in India: Vulnerability assessment, food security measures and policy recommendation. *Sci Total Environ* 849:157850

4. Abdirad S et al (2022) Proteomic analysis of the meristematic root zone in contrasting genotypes reveals new insights in drought tolerance in rice. *Proteomics*, 22:00100
5. Chávez-Arias CC et al (2021) Maize responses challenged by drought, elevated daytime temperature and arthropod herbivory stresses: A physiological, biochemical and molecular view. *Frontiers in Plant Science*, 15:12
6. Kang J et al (2022) Antioxidant Metabolism Underlies Different Metabolic Strategies for Primary Root Growth Maintenance under Water Stress in Cotton and Maize. *Antioxidants* 11, 820
7. Ali S et al (2022) Pragmatic role of microbial plant biostimulants in abiotic stress relief in crop plants. *J Plant Interact* 17:705–718
8. Vijayaraghavareddy P et al (2022) Production and scavenging of reactive oxygen species confer to differential sensitivity of rice and wheat to drought stress. *Crop and Environment* 1:15–23
9. Liu R et al (2022) Oxidative Stress in Cancer Immunotherapy: Molecular Mechanisms and Potential Applications. *Antioxidants* 11:853
10. Fadji AE et al (2022) Efforts towards overcoming drought stress in crops: Revisiting the mechanisms employed by plant growth-promoting bacteria. *Frontiers in Microbiology* 13
11. Satti SH et al (2022) Plant-Based Titanium Dioxide Nanoparticles Trigger Biochemical and Proteome Modifications in *Triticum aestivum* L. under Biotic Stress of *Puccinia striiformis*. *Molecules* 27:4274
12. Jangir P et al (2021) Role of *Serendipita indica* in enhancing drought tolerance in crops. *Physiol Mol Plant Pathol* 116:101691
13. Manna M et al (2021) Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. *Physiol Plant* 172:847–868
14. Wu J et al (2022) Physiology of Plant Responses to Water Stress and Related Genes: A Review. *Forests* 13:324
15. Mamik S, Sharma AD (2014) Analysis of boiling stable antioxidant enzymes in invasive alien species of *Lantana* under abiotic stress-like conditions. *Brazilian J Bot* 37:129–141
16. Nischal P, Sharma AD (2020) Stomatal and pollen dependant metabolic changes as a metric of stress tolerance and invasive potential of invasive plant–*Lantana camara* (L.) growing under abiotic stress like conditions. *South Afr J Bot* 131:406–420
17. Abreha KB et al (2022) Sorghum in dryland: morphological, physiological, and molecular responses of sorghum under drought stress. *Planta* 255:1–23
18. Bukhari MA et al (2022) Enhancing Drought Stress Tolerance in *Camelina* (*Camelina sativa* L.) through Exogenous Application of Potassium. *Physiologia Plantarum*, e13779
19. Barmukh R et al (2022) Characterization of “QTL-hotspot” introgression lines reveals physiological mechanisms and candidate genes associated with drought adaptation in chickpea. *Journal of Experimental Botany*
20. Guo X et al (2022) Role of WRKY Transcription Factors in Regulation of Abiotic Stress Responses in Cotton. *Life* 12, 1410

21. Han J et al (2022) Evolution of the DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN subfamily in green plants. *Plant Physiol* 190:421–440
22. Bublyk O et al (2022) Comparative Analysis of Promoters of DREB2B Transcription Factor Genes in *Deschampsia antarctica* and Other Grasses. *Cytol Genet* 56:399–409
23. Meena RP et al (2022) Expression of a *Pennisetum glaucum* gene DREB2A confers enhanced heat, drought and salinity tolerance in transgenic *Arabidopsis*. *Molecular Biology Reports*, 1–12
24. Figueroa N, Gómez R (2022) Bolstered plant tolerance to low temperatures by overexpressing NAC transcription factors: identification of critical variables by meta-analysis. *Planta* 256:1–13
25. Toppino L et al (2022) Next Generation Breeding for Abiotic Stress Resistance in Eggplant. *Genomic Designing for Abiotic Stress Resistant Vegetable Crops*. Springer, pp 115–151
26. Karmacharya A (2022) Molecular Mapping of QTL for Genetic Transformation-Related Trait and CRISPR/Cas9-Mediated Gene Editing in Wheat. North Dakota State University
27. Liu J et al (2022) Shaping polyploid wheat for success: Origins, domestication, and the genetic improvement of agronomic traits. *J Integr Plant Biol* 64:536–563
28. Abbas A et al (2021) Genetic diversity and synergistic modulation of salinity tolerance genes in *Aegilops tauschii* Coss. *Plants* 10:1393
29. Yu H et al (2021) Distribution, Genetic Diversity and Population Structure of *Aegilops tauschii* Coss. in Major Wheat-Growing Regions in China. *Agriculture* 11:311
30. Abbas A et al (2021) Selection and Validation of Reference Genes for RT-qPCR Analysis in *Aegilops tauschii* (Coss.) under Different Abiotic Stresses. *Int J Mol Sci* 22:11017
31. Leigh FJ et al (2022) Progenitor species hold untapped diversity for potential climate-responsive traits for use in wheat breeding and crop improvement. *Heredity* 128:291–303
32. Hao Y et al (2022) Genome-wide survey of the dehydrin genes in bread wheat (*Triticum aestivum* L.) and its relatives: identification, evolution and expression profiling under various abiotic stresses. *BMC Genomics* 23:1–18
33. Custodio-Mendoza J et al (2022) GC–MS determination of malondialdehyde, acrolein, and 4-hydroxy-2-nonenal by ultrasound-assisted dispersive liquid-liquid microextraction in beverages. *Food Chem* 384:132530
34. Hajhashemi S, Ehsanpour A (2013) Influence of exogenously applied paclobutrazol on some physiological traits and growth of *Stevia rebaudiana* under in vitro drought stress. *Biologia* 68:414–420
35. Laguerre M et al (2008) Conjugated autoxidizable triene (CAT) assay: a novel spectrophotometric method for determination of antioxidant capacity using triacylglycerol as ultraviolet probe. *Anal Biochem* 380:282–290
36. Abbas A et al (2020) Assessment of the genetic diversity in *Aegilops tauschii* (coss.) By using ssr markers and morphophysiological traits. *Appl Ecol Environ Res* 18:7011–7020

37. Zhang C et al (2007) Alert and prevention of the spreading of *Aegilops tauschii*, a worst weed in wheat field. *Acta Phytolacica Sinica* 34:103–106
38. Itam M et al (2020) *Aegilops tauschii* introgressions improve physio-biochemical traits and metabolite plasticity in bread wheat under drought stress. *Agronomy* 10:1588
39. Bhanse P et al (2022) Role of plant growth-promoting rhizobacteria in boosting the phytoremediation of stressed soils: Opportunities, challenges, and prospects. *Chemosphere*,134954
40. Shamuyarira KW et al (2022) A meta-analysis of combining ability effects in wheat for agronomic traits and drought adaptation: Implications for optimizing biomass allocation. *Crop Sci* 62:139–156
41. Pour-Aboughadareh A et al (2022) Association Analysis for Some Biochemical Traits in Wild Relatives of Wheat under Drought Stress Conditions. *Genes* 13:1491
42. Gerbi H et al (2022) Physiological drought resistance mechanisms in wild species vs. rootstocks of almond and plum. *Trees* 36:669–683
43. Alharbi K et al (2022) Impact of *Plantago ovata* Forsk leaf extract on morpho-physio-biochemical attributes, ions uptake and drought resistance of wheat (*Triticum aestivum* L.) seedlings. *Front Plant Sci* 13:999170
44. Liang J et al (2022) Identification of PP2C Genes in Tibetan Hulless Barley (*Hordeum vulgare* var. *nudum*) Under Dehydration Stress and Initiatory Expression and Functional Analysis of HvPP2C59. *Plant Molecular Biology Reporter*,1–17
45. Suárez JC et al (2022) Chlorophyll Fluorescence Imaging as a Tool for Evaluating Disease Resistance of Common Bean Lines in the Western Amazon Region of Colombia. *Plants* 11:1371
46. Li Y et al (2022) Exogenous SA or 6-BA maintains photosynthetic activity in maize leaves under high temperature stress. *The Crop Journal*
47. Arian B et al (2022) Fe₂O₃-modified graphene oxide mitigates nanoplastic toxicity via regulating gas exchange, photosynthesis, and antioxidant system in *Triticum aestivum*. *Chemosphere* 307:136048
48. Ali B et al (2022) Salt tolerance screening of a newly developed wheat variety (AZRC-DK-84) in saline environment using halophytic grass (*Cenchrus penisetiformis*) as a test model. *Acta Physiol Plant* 44:1–16
49. Grehan J (2022) Medical Officers on the Infamous Burma Railway: Accounts of Life, Death and War Crimes by Those Who Were There With F-Force. *Medical Officers on the Infamous Burma Railway*,1–224
50. Ahmad A et al (2022) Screening of wheat (*Triticum aestivum* L.) genotypes for drought tolerance through agronomic and physiological response. *Agronomy* 12:287
51. Minaei S et al (2022) High genetic diversity in *Aegilops tauschii* Coss. accessions from North Iran as revealed by IRAP and REMAP markers. *J Genetic Eng Biotechnol* 20:1–12
52. Na C et al (2022) Exogenously applied Spd and Spm enhance drought tolerance in tea plants by increasing fatty acid desaturation and plasma membrane H⁺-ATPase activity. *Plant Physiol Biochem*

53. Sun W et al (2020) Adaptation of acupuncture and traditional Chinese herbal medicines models because of climate change. *Journal of Stress Physiology & Biochemistry* 16
54. Xu J et al (2022) Physiological, biochemical and metabolomic mechanisms of mitigation of drought stress-induced tobacco growth inhibition by spermidine. *Ind Crops Prod* 181:114844
55. Mukarram M et al (2021) Drought: Sensing, signalling, effects and tolerance in higher plants. *Physiol Plant* 172:1291–1300
56. Li L, Yi H (2022) Enhancement of drought tolerance in Arabidopsis plants induced by sulfur dioxide. *Ecotoxicology*, 1–12
57. Seckin H et al (2022) An environmental approach for the photodegradation of toxic pollutants from wastewater using Pt–Pd nanoparticles: Antioxidant, antibacterial and lipid peroxidation inhibition applications. *Environmental Research*, 112708
58. Zhang L et al (2022) Brassinolide alleviated chilling injury of banana fruit by regulating unsaturated fatty acids and phenolic compounds. *Sci Hort* 297:110922
59. Guan G et al (2021) Antibacterial properties and mechanism of biopolymer-based films functionalized by CuO/ZnO nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. *J Hazard Mater* 402:123542
60. Zhou M et al (2021) γ -Aminobutyric Acid (GABA) Priming Improves Seed Germination and Seedling Stress Tolerance Associated With Enhanced Antioxidant Metabolism, DREB Expression, and Dehydrin Accumulation in White Clover Under Water Stress. *Front Plant Sci* 12:776939
61. Rustamova S et al (2021) Expression profiling of DREB1 and evaluation of vegetation indices in contrasting wheat genotypes exposed to drought stress. *Plant Gene* 25:100266
62. Wang Y et al (2022) Overexpression of a tomato AP2/ERF transcription factor SIERF. B1 increases sensitivity to salt and drought stresses. *Sci Hort* 304:111332
63. Jiang Q et al (2022) Genome-wide identification and characterization of AP2/ERF gene superfamily during flower development in *Actinidia eriantha*. *BMC Genomics* 23:1–16
64. Mei F et al (2022) A gain-of-function allele of a DREB transcription factor gene ameliorates drought tolerance in wheat. *The Plant Cell*
65. Pour-Aboughadareh A et al (2020) Wild relatives of wheat respond well to water deficit stress: A comparative study of antioxidant enzyme activities and their encoding gene expression. *Agriculture* 10:415

Figures

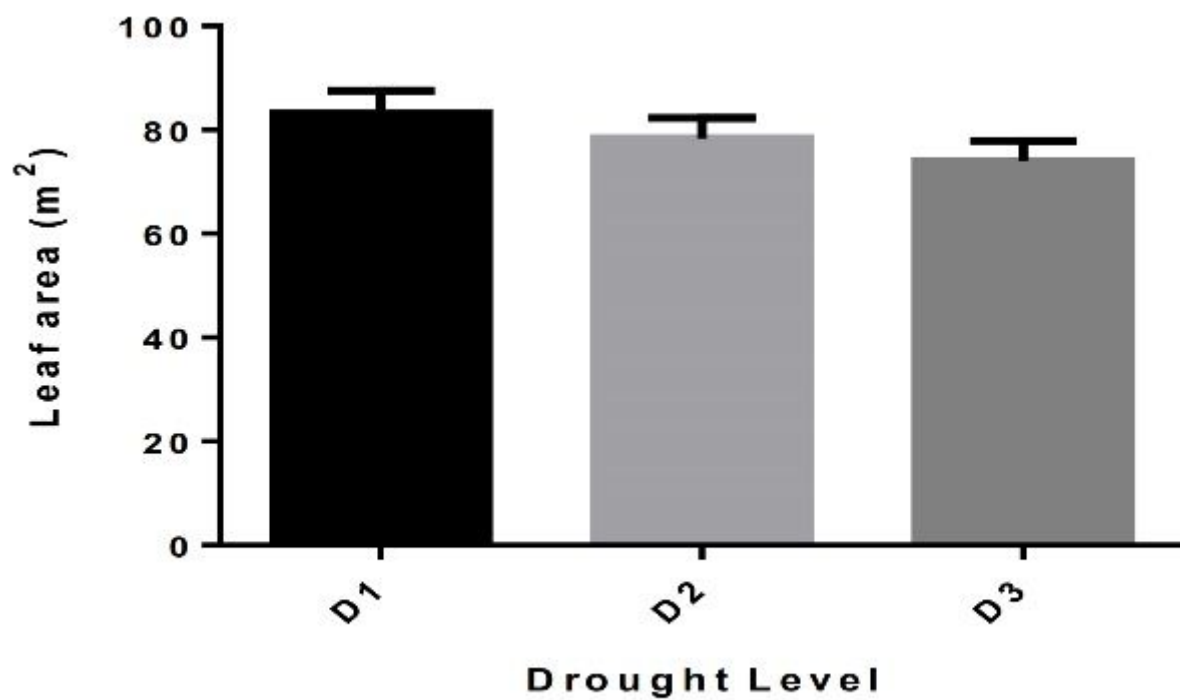


Figure 1

Effect of different drought stress levels on leaf area (m²) of *Ae. tauschii*. (wheres D₁= 15-25%, D₂= 45-55% and D₃= 75-85%).

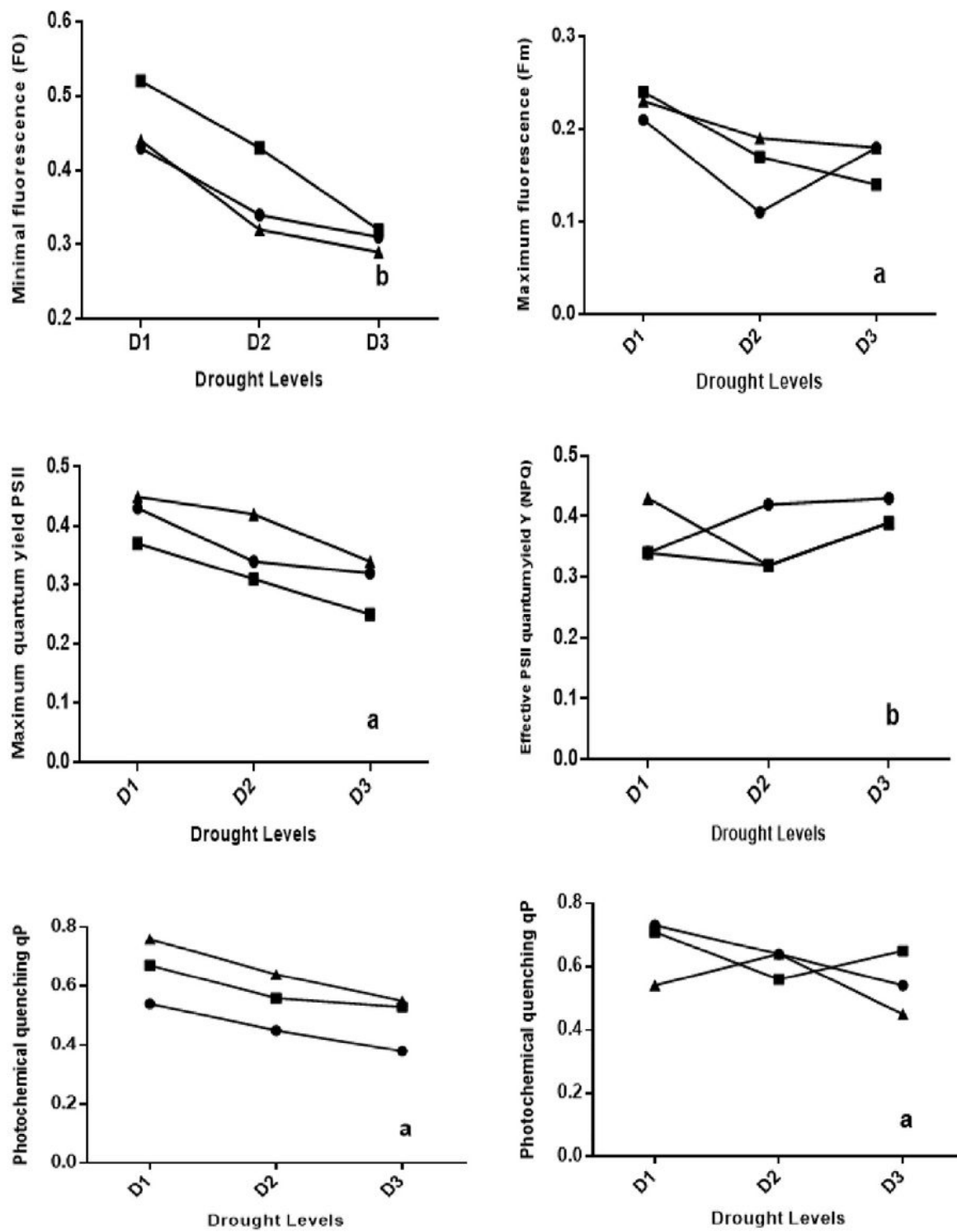


Figure 2

Effect of different drought stress level on different photosynthesis parameters.

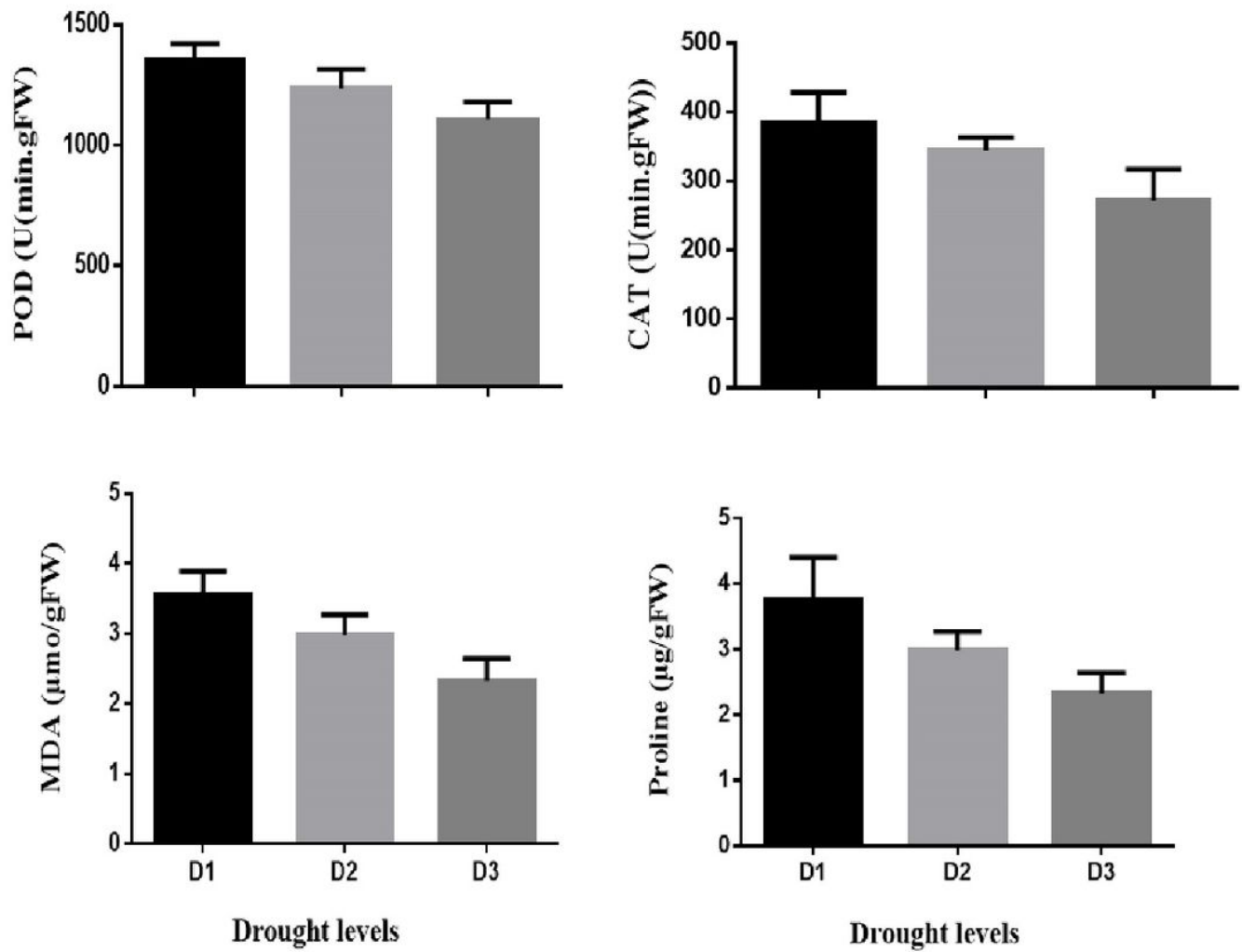


Figure 3

Effects of different drought stress levels on peroxidase (A), catalase (B), proline (C), and malondialdehyde (D) in *Ae. tauschii*.

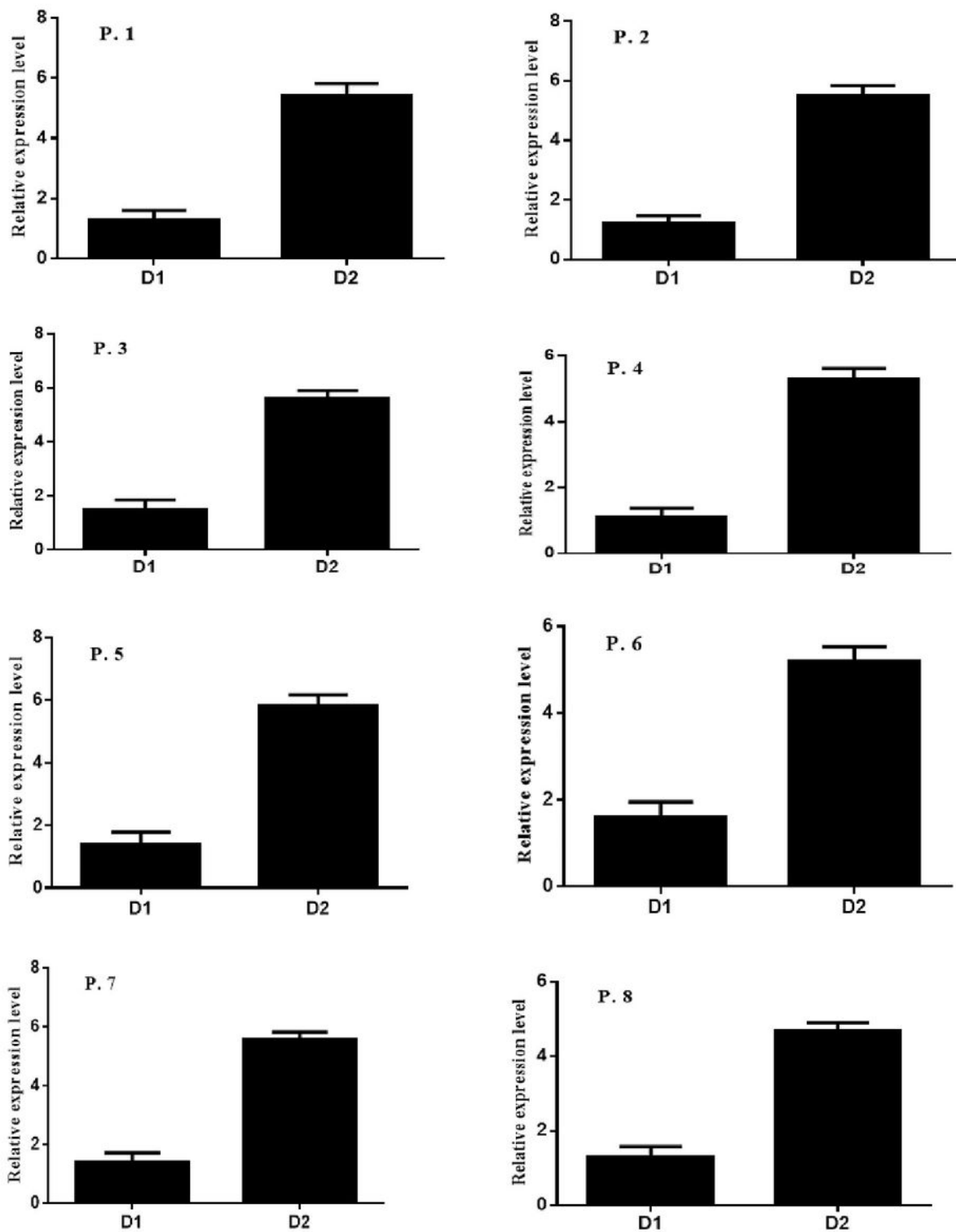


Figure 4

Relative expression levels of DREB in *Ae. tauschii* populations under different drought stress conditions (75 g L⁻¹ PEG-6000). Actin was used as an internal reference. Experiments were repeated three times. Values are means, and bars indicate standard deviation (S.D.).