Duan S *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 2, Article number 14338
DOI:10.15835/nbha53214338
Research Article

Effects of drought stress on seed germination, growth and physiological traits of dwarf wheats at seedling and maturity stage

Shan DUAN[1,2,3,4*], Peipei AN[1,2,3,4], Xiaoyan ZHANG[1,2,3,4], Xuezhen LIU[1], Jia ZHANG[1], Xiaoyu WANG[1], Lei XING[1], Julian ZHAO[1,2,3,4]

*[1]Longdong University, School of Agriculture and Bioengineering, No. 45 Lanzhou Road, Xifeng District, Qingyang, Gansu, 745000, China; 1207187440@qq.com (SD) (*corresponding author) peipeian@ldxy.edu.cn (PAN); 1226573428@qq.com (XZ); 1683786245@qq.com (XL); 3408382224@qq.com (JZ); 2739533263@qq.com (XW); 2731497384@qq.com (LX); gsqyzjl@126.com (JZ)*
[2]Longdong University, Qingyang, Gansu Dryland Research Center of winter wheat germplasm innovation and Application Engineering, No. 45 Lanzhou Road, Xifeng District, Qingyang, Gansu, 745000, China
[3]Longdong University, Qingyang, Gansu Collaborative Innovation of Academicians and Experts on Dryland Agriculture in the Loess Plateau, No. 45 Lanzhou Road, Xifeng District, Qingyang, Gansu, 745000, China
[4]Longdong University, Qingyang, Collaborative Innovation Center for Longdong Dryland Crop Germplasm Improvement and Industrialization, No. 45 Lanzhou Road, Xifeng District, Qingyang, Gansu, 745000, China

---

Abstract

Water deficiency is a major constraint in wheat productivity in arid and semi-arid areas. Height-reducing genes, such as *Rht* decrease plant height while enhancing yield. However, their benefits in arid environments are poorly understood. We evaluate the effects of drought induced by 10% and 20% polyethylene glycol (PEG) 6000, and water shortage on seed germination, growth, physiological traits, and agronomic traits of 12 wheat varieties (11 dwarf and 1 tall) at seedling and maturity stages. Several indicators (germination energy, germination index, root number, and maximum root, coleoptile, and shoot lengths) in some-varieties were promoted by 10% PEG-6000, however, all indicators were inhibited at 20% PEG-6000. A positive and significant correlation occurred between shoot fresh weight and both shoot dry weight and relative water content. Shoot fresh weight explained most (positive) variation in principal component analysis (PC1), and peroxidase activity was the least (negative) variation. Plant height and yield components decreased in all wheat varieties with moderate and severe drought stress. A drought resistance coefficient revealed *rht* (Langdon) was most drought resistant at 10% PEG-6000, *Rht2* (Xinong 223) at 20% PEG-6000, and *Rht8* (Jinmai 47) at both moderate and severe drought stress. These findings can be used to identify appropriate wheat varieties to cultivate in water-deficient areas.

Keywords: different dwarf genotypes; drought resistance coefficient; hydroponic experiments; pot experiment; water stress

---

Abbreviations

Rht: Height-reducing genes; PEG-6000: polyethylene glycol 6000; GA: Gibberellin; ROS: Reactive oxygen species; SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxide; POD: peroxidase; MDA: malondialdehyde; GR: germination rate; CK: control; GE:

Received: 26 Dec 2024. Received in revised form: 10 Jun 2025. Accepted: 19 Jun 2025. Published online: 30 Jun 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Germination energy; DI: Germination drought-resistant index; RWC: Relative water content; GI: Germination index; PCA: Principal component analyses; SPAD: Soil and Plant Analyzer Development

---

Introduction

Wheat is the primary staple crop cultivated in arid and semi-arid regions. However, climate change has intensified drought severity, and significantly reduced wheat yield. Between 1950 and 2021 in China, an area of 142,312,540,000 hm^2 was affected by drought, causing a grain loss of 1,162,190,000 t (Xu *et al.*, 2024). Wheat is highly sensitive to drought during its nutritional growth stage, and the vigor of wheat seedlings can affect growth and ultimately yield (Daryanto *et al.*, 2017). To ensure stable wheat yields in drought conditions, the identification of wheat drought-resistant breeds and water-saving germplasm is necessary. Therefore, the characterization of drought tolerance in wheat during the seedling stage will facilitate the identification of drought-tolerant varieties and their breeding technology (Mohi-Ud-Din *et al.*, 2021).

Wheat grain yield increased considerably when plant height reduced in the 1960s following the introduction of the dwarf genes *Rht-B1b* and *Rht-D1b* in what was named the "Green Revolution." As of 2000, more than 70% of commercial wheat cultivars worldwide contained *Rht-B1b* and *Rht-D1b*. Most wheat grown in the North China winter and Yellow and Huai Valley facultative wheat regions contains *Rht-B1b* and *Rht-D1b*; *Rht8* is also widely used in the northwestern spring and southwestern China autumn-sown wheat regions (Hedden, 2003; Zhang *et al.*, 2006).

Wheat containing dwarf genes (e.g., *Rht8*, *Rht13*) can increase grain yield due to improved water-use efficiency in arid environments. In drought conditions, when two dwarf genes are combined (e.g., *Rht8*+*Rht-B1b*, *Rht8*+*Rht-D1b*), both water-use efficiency and yield increase (Liu *et al.*, 2017; Rebetzke *et al.*, 2012). Coleoptile length is an important indicator of wheat seed drought tolerance; shorter coleoptiles have poor emergence rates when cultivars are sown deeper to access deep-water reserves in arid and semi-arid regions (Radford, 1987). Dwarf genes insensitive to gibberellin (GA) (*Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-D1c*, *Rht11*, and *Rht17*) have significantly shorter coleoptile lengths, while there are no negative effects of other dwarf genes sensitive to GA (*Rht4*, *Rht5*, *Rht8*, *Rht12–15*, *Rht18*, and *Rht24*) on coleoptile length; however, the effects of dwarf genes (insensitive or sensitive to GA) on seedling vigor are similar to those on coleoptile length (Rebetzke and Richards, 2000; Ellis *et al.*, 2004; Wang *et al.*, 2015; Duan *et al.*, 2020).

Drought stress can negatively affect plant growth, morphology, physiology, and yield. These effects depend on crop developmental stage, and the severity and duration of stress, and they can also induce an increase in osmotic substances, modify phytohormone and chlorophyll contents, and accumulate reactive oxygen species (ROS) (Ihsan *et al.*, 2016; Park *et al.*, 2025). Plants have evolved diverse strategies to cope with drought stress, including production of several enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX), and peroxidase (POD) to remove the ROS, and accumulation of sugars, proline, betaine, trehalose, and fructan to promote water retention (Li *et al.*, 2010; Farooq *et al.*, 2014). Additionally, malondialdehyde (MDA), a byproduct of lipid peroxidation, is a main indicator of oxidative damage, reflecting drought stress tolerance (Møller *et al.*, 2007).

Wheat can adapt to drought stress by adjusting its morphology. Seedlings can improve drought resistance by changing biomass allocation to increase root specific gravity, and physiological changes in the root system. A significant positive correlation between wheat seedling biomass and drought resistance indicates that high seedling vigor produces strong drought resistance (Liu *et al.*, 2020; Pour-Aboughadareh *et al.*, 2020). Morphological traits such as seed germination or germination rate (GR), number of primary radicles, length of maximum primary radicle, and coleoptile length have been used to characterize drought tolerance in wheat at seed germination or seedling stages, and plant height, flag leaf length and width, spike length, and 1000-kernel weight can be used to identify drought resistance at maturity (Sallam *et al.*, 2019; Mahpara *et al.*, 2022).

We aim to report the impacts of polyethylene glycol (PEG) 6000 on germination and seedling traits of different dwarf genes to identify which of the 12 genotypes performs best in growth and yield. We use drought tolerance indices to determine the optimal index, and to evaluate agronomic traits at maturity under water deficiency to evaluate the drought resistance of dwarf genes.

Materials and Methods

Experimental materials, and germination and hydroponic culture conditions

Materials, including 11 dwarf genes and Langdon (no known dwarf gene, used as a control) were provided by Professor Yingang Hu, College of Agronomy, Northwest A&F University (Table 1). Plump, pest- and disease-free seeds were disinfected for 8 min with 0.1% $KMnO_4$, repeatedly rinsed with distilled water, and then placed in Petri dishes covered with two layers of filter paper. A sample of 50 seeds was placed into each Petri dish, and 10 mL of polyethylene glycol (PEG) 6000 (10% or 20%) was added for treatment; an equal amount of distilled water was added as a control for each genotype (water level was marked on the outside of each dish). Three biological replicates were established for each treatment, and all Petri dishes were placed into an incubator for germination and incubation for 7 days. Cultivation conditions involved 20 °C, 14 h/10 h (day/night), and a relative humidity of 80%. Water was added to the marked level of each Petri dish every other day because the plant does not absorb PEG-6000; numbers of germinated seeds were recorded daily. We considered seeds to have begun germination at a bud length of 50% seed length; when no further seeds had germinated within each of three replicate Petri dishes within a treatment over three consecutive days, germination was considered to have ended for that treatment. The number of germinations day^{-1} and the total number of germinations were counted.

Table 1. The names and Gibberellin (GA) sensitivity of dwarf genes in wheat

Number	Wheat Varieties	Dwarf genes	GA sensitivity
1	Langdon	*rht*	-
2	Xiaoyan 6	*Rht1*	insensitivity
3	Xinong 223	*Rht2*	insensitivity
4	Burt ert 937	*Rht4*	sensitivity
5	Marfed M	*Rht5*	sensitivity
6	Jinmai 47	*Rht8*	sensitivity
7	Granato	*Rht9*	sensitivity
8	Karcagi	*Rht12*	sensitivity
9	Magnif M1	*Rht13*	sensitivity
10	Castelporziano	*Rht14*	sensitivity
11	Durox	*Rht15*	sensitivity
12	Icaro	*Rht18*	sensitivity

A sample of 100 sterilized seeds was placed into a plastic box (20 × 15 cm) containing Hoagland nutrient solution and transferred to a light incubator. Wheat seedlings at the “one leaf and one heart” stage were treated with Hoagland nutrient solution containing 10% or 20% PEG-6000; no PEG stress was used as a control (CK). The experiment ran for 10 d; treatments were replicated thrice.

Measurement items and methods

Seed germination rate

Germination rate, germination energy, germination index, and the germination drought-resistant index of seeds were calculated using formulae 1-4 (Li *et al.*, 2020b).

$$\text{Germination rate (GR\%)} = \frac{\text{No. of germinated seeds}}{\text{No. of tested seeds}} * 100 \quad (1)$$

$$\text{Germination energy (GE\%)} = \frac{\text{Maximum daily No. of germinated seeds}}{\text{No. of tested seeds}} * 100 \quad (2)$$

$$\text{Germination index (GI)} = \sum \frac{Gt}{\text{Dt}} \quad (3)$$

Where G_t is the number of germinations on day *t*, and D_t is the number of germination days at the corresponding time.

$$\text{Germination drought} - \text{resistant index (DI))} = \frac{\text{Germination index under treatment}}{\text{Germination index under control}} \quad (4)$$

Seedling shoot and root growth indices

On day 7 following PEG-6000 treatment, 5 similarly sized plants were selected to record maximum root length, root number, coleoptile length, seedling height, and shoot and root fresh weights. Plants were then subjected to high-temperature desiccation at 105 °C for 0.5 h, then dried at 80 °C to constant weight, dry shoot and root weights were recorded.

Physiological indices of seedling leaves

On day 10 following PEG-6000 treatment, the relative water content (RWC) of three plants from each replicate was determined following Saddiq *et al.* (2021).

$$\text{Relative water content (RWC)} = \left(\frac{\text{Wf}-\text{Wd}}{\text{Ws}-\text{Wd}}\right) * 100 \quad (5)$$

Where Wf represents fresh leaf weight, Ws represents the weight of saturated leaves, and Wd represents dry weight of saturated leaves.

Chlorophyll content was calculated following Nagata *et al.* (1992); malondialdehyde (MDA) content was measured using the thiobarbituric acid color method (Lei *et al.*, 2014); proline content was assayed using acidic-ninhydrin (Bates *et al.*, 1973); soluble protein content was estimated based on Coomassie brilliant blue G-250 staining with a standard curve of bovine serum albumin (Bradford, 1976); soluble sugar content was determined using the anthrone colorimetric method (Buysse and Merckx, 1993); and superoxide dismutase (SOD) and peroxidase (POD) activities were examined following Li *et al.* (2022)

Pot experiments

Seeds were planted in pots (20 cm diameter, 18 cm height) to examine drought tolerance within a greenhouse (with 10 kg soil). Experimental soil organic matter, total nitrogen, and available phosphorus and potassium contents were 17.8 g kg^{-1}, 0.96 g kg^{-1}, 18.75 mg kg^{-1}, and 251.8 mg kg^{-1}, respectively. Fertilizer (5.5×10^{-4} kg urea ($N \geq 46.4\%$), 2.79×10^{-3} kg superphosphate ($P_2O_5 \geq 12\%$), 8.6×10^{-4} kg potassium chloride ($K_2O \geq 40\%$)) was applied to each pot; a further 5.5×10^{-4} kg urea was applied at the jointing stage. Two drought treatment levels were established: normal moisture treatment (soil relative moisture content 75 ± 5%), moderate drought (soil relative moisture content 50 ± 5%), and severe drought (soil relative moisture content 35 ± 5%). Soil relative water content was measured using a TDR300 portable soil moisture meter (Spectrum, USA). Each treatment was replicated five times (5 pots), with 5 plants per pot. The drought treatment was performed at the heading stage, with pots watered every 2 or 3 d; the amount of water pot^{-1} was calculated based on soil moisture content; a measuring cylinder was used for volume calculation. Physiological indices, SPAD (Soil and Plant Analyzer Development) value, and the area of flag leaves were measured at the filling stage. All plants were harvested at the maturation stage (GS91). Plant height and yield components (spike length, spikelet number $spike^{-1}$, grain number $spike^{-1}$, and 1000-kernel weight) were determined after harvest.

Statistical analysis

Subordinate function value analysis was used to estimate the drought resistance of dwarf genes, calculated using equation 6 (Zhang *et al.*, 2011):

$$U(Xj) = \frac{Xj - Xmin}{Xmax - Xmin}, j = 1, 2, 3, \dots, n \qquad (6)$$

Where X_j is the subordinate function value of the jth indicator; Xj denotes the value of the jth drought resistance coefficient; and X_{min} and X_{max} represent the minimum and maximum values of the jth indicator drought resistance coefficient, respectively.

The weight of each comprehensive index (W) was determined using equation 7 (Zhang *et al.*, 2011):

$$Wj = \frac{Pj}{\sum_{j=1}^{n} Pj} \qquad (7)$$

Where W_j is the weight of the jth indicator among all indicators; and P_j is the contribution rate of the jth indicator.

The comprehensive drought resistance coefficient D value was calculated using equation 8 (Zhang *et al.*, 2011):

$$D = \sum_{j=1}^{n} [U(Xj) * Wj], j=1, 2, 3,\dots, n \qquad (8)$$

Statistical analyses (variance, correlation, Duncan's multiple comparison, and principal components) were performed using IBM SPSS Statistics 20.0. Figures were created using Origin 2021.

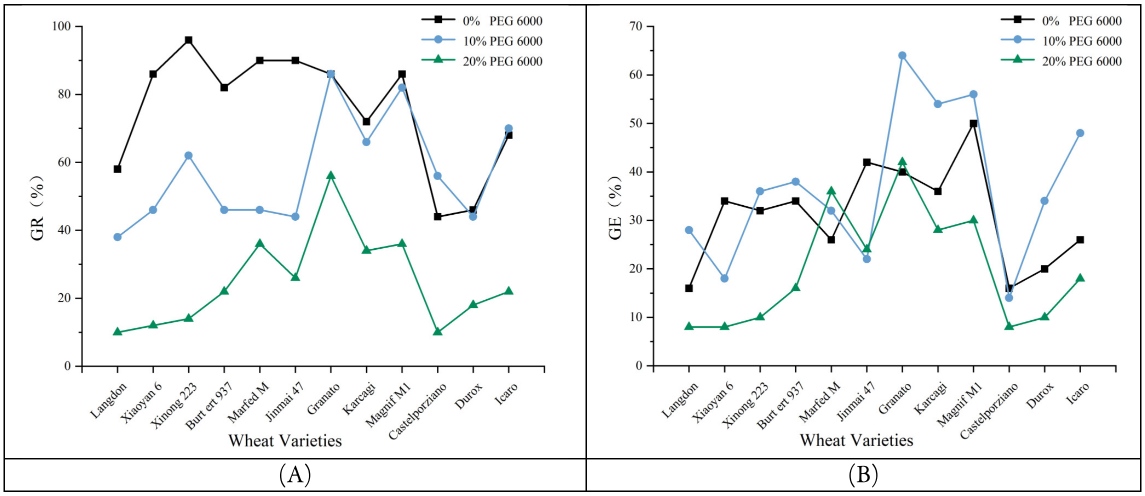
Results

Seed germination traits in PEG-6000 treatments

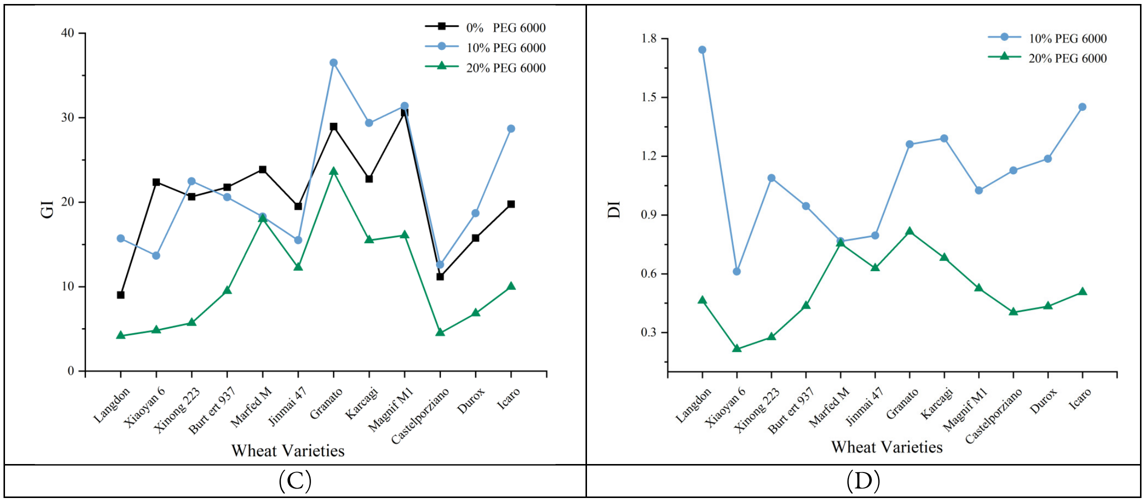
The germination rate (GR) of the 12 wheat varieties decreased after treatment with 10% and 20% PEG-6000, excepting *Rht9* (no reduction) and *Rht18* (a slight increase) at 10% polyethylene glycol (PEG) 6000 (Figure 1A). Germination energy (GE) of most wheat varieties (*rht*, *Rht2*, *Rht4*, *Rht5*, *Rht9*, *Rht12*, *Rht13*, *Rht15*, *Rht18*) was promoted by 10% PEG-6000, but reduced at 20% PEG-6000 (excepting *Rht5* and *Rht9*) (Figure 1B). The germination index (GI) exhibited similar trends with GE when treated with PEG-6000 (Figure 1C). The germination drought-resistant index (DI) decreased with increased PEG-6000 concentration, with the lowest reduction for *Rht5* (Figure 1D).

Seedling shoot and root traits with PEG-6000 treatment

Changes in seedling shoot and root traits differed between varieties (Tables 2, 3; **S1, S2**). Compared with controls, root numbers for *rht*, *Rht2* and *Rht4* increased slightly at 10% PEG-6000, but decreased significantly in all varieties at 20% PEG-6000. Furthermore, 10% PEG-6000 accelerated growth of maximum root, coleoptile, and shoot lengths, but at 20% PEG-6000 inhibited their growth, significantly so for *Rht12*.



(A) (B)



(C) (D)

Figure 1. Effects of 10% and 20% PEG-6000 on: A) germination rate (GR); B) germination energy (GE); C) germination index (GI); and D) germination drought-resistant index (DI) of 12 wheat varieties

Table 2. Effects of 10% and 20% PEG-6000 on root number, and maximum root, coleoptile, and shoot lengths for 12 wheat varieties

Wheat Varieties	**Root number**			**Max root length (cm)**			**Coleoptile length (cm)**			**Shoot length (cm)**		
	0% PEG-6000	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**
Langdon	4.50 ± 0.67 de	4.90 ± 0.30 bc	3.20 ± 0.40 cd	11.58 ± 1.50 cd	14.00 ± 1.80 def	5.04 ± 1.40 abc	3.79 ± 0.30 a	5.02 ± 3.21 a	1.94 ± 1.06 abc	12.18 ± 1.82 fg	15.83 ± 3.50 a	10.16 ± 1.86 bcd
Xiaoyan 6	5.00 ± 0.77 cd	4.80 ± 0.60 bcd	3.40 ± 0.80 bcd	16.45 ± 1.60 a	14.73 ± 2.30 cde	6.22 ± 1.20 ab	3.79 ± 0.26 a	3.75 ± 0.64 bc	1.64 ± 0.64 bc	15.87 ± 3.36 bcd	13.30 ± 2.53 bcd	9.64 ± 0.44 cde
Xinong 223	4.10 ± 0.70 ef	4.70 ± 0.78 bcd	3.80 ± 0.40 abc	13.21 ± 0.90 bc	15.15 ± 1.10 cd	4.68 ± 0.50 abcde	2.44 ± 0.17 d	2.94 ± 0.20 c	2.36 ± 0.22 ab	9.95 ± 1.07 h	13.76 ± 0.97 abc	8.14 ± 1.13 g
Burt ert 937	4.30 ± 0.78 def	4.80 ± 0.40 bcd	3.40 ± 0.73 bcd	16.26 ± 2.20 a	16.10 ± 1.10 bc	6.60 ± 0.70 a	2.81 ± 0.33 a	3.91 ± 0.95 bc	2.60 ± 0.20 a	14.10 ± 1.70 cdef	13.83 ± 1.30 abc	9.32 ± 0.69 def
Marfed M	4.50 ± 0.50 de	4.20 ± 0.60 d	3.80 ± 0.40 abc	17.74 ± 2.00 a	18.36 ± 1.50 a	4.96 ± 0.60 abcd	3.32 ± 0.31 b	3.49 ± 0.52 bc	2.32 ± 0.21 ab	15.50 ± 2.71 bcde	15.84 ± 1.78 a	10.10 ± 0.46 bcde
Jinmai 47	4.70 ± 0.90 de	4.40 ± 0.49 cd	4.20 ± 0.40 abc	11.63 ± 1.90 cd	12.20 ± 1.20 gh	5.74 ± 1.30 abc	3.11 ± 0.24 bc	2.90 ± 0.48 c	2.52 ± 0.19 a	13.65 ± 3.39 efg	12.77 ± 2.58 cd	9.72 ± 0.63 cde
Granato	6.70 ± 1.10 a	5.30 ± 0.64 ab	4.80 ± 0.75 a	12.46 ± 1.50 bc	11.93 ± 1.70 gh	5.54 ± 1.80 abc	3.98 ± 0.38 a	4.48 ± 0.34 ab	1.94 ± 0.45 abc	14.08 ± 0.97 cdef	15.61 ± 1.63 ab	10.94 ± 0.45 ab
Karcagi	3.60 ± 0.80 f	3.40 ± 0.80 e	2.40 ± 0.49 d	13.72 ± 1.00 b	12.17 ± 1.90 gh	3.60 ± 0.30 cde	2.97 ± 0.20 c	2.97 ± 0.28 c	1.26 ± 0.69 c	13.88 ± 0.69 defg	11.39 ± 1.09 d	8.26 ± 0.69 g
Magnif M1	4.90 ± 0.30 d	4.50 ± 0.50 cd	4.20 ± 0.40 abc	16.28 ± 1.70 a	17.50 ± 1.40 ab	4.30 ± 0.20 bcde	3.03 ± 0.12 bc	3.26 ± 0.20 c	2.30 ± 0.24 ab	18.63 ± 1.58 a	15.50 ± 2.55 ab	11.58 ± 0.12 a
Castelporziano	5.70 ± 0.78 bc	5.60 ± 0.49 a	4.20 ± 0.75 abc	10.51 ± 1.90 d	11.58 ± 1.80 h	2.80 ± 1.00 de	3.04 ± 0.36 bc	3.77 ± 0.33 bc	1.98 ± 0.16 abc	11.91 ± 1.83 g	12.29 ± 2.44 cd	8.58 ± 0.16 fg
Durox	5.00 ± 0.63 cd	4.80 ± 0.87 bcd	3.20 ± 0.98 cd	12.41 ± 1.10 bc	12.66 ± 1.40 fgh	3.52 ± 0.60 cde	3.81 ± 0.31 c	3.95 ± 0.46 bc	1.92 ± 0.25 abc	16.06 ± 1.70 bc	13.81 ± 2.35 abc	9.92 ± 0.25 cde
Icaro	6.20 ± 0.60 ab	5.50 ± 0.50 a	4.40 ± 0.80 ab	13.92 ± 2.00 b	13.39 ± 1.50 efg	2.72 ± 1.60 e	4.01 ± 0.41 a	3.97 ± 0.53 bc	1.36 ± 0.76 c	17.05 ± 1.57 ab	14.52 ± 1.65 abc	10.36 ± 0.76 bc

Values are means ± SD (standard deviations). Means with different letters within a column indicate significant differences (Duncan's test, $p \leq 0.05$).

Table 3. Effects of 10% and 20% PEG-6000 on fresh and dry root and shoot weights for 12 wheat varieties

Wheat Varieties	**Root fresh weight (g)**			**Shoot fresh weight (g)**			**Root dry weight (g)**			**Shoot dry weight (g)**		
	0% PEG-6000	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**	**0% PEG-6000**	**10% PEG-6000**	**20% PEG-6000**
Langdon	0.34 ± 0.03 abc	0.25 ± 0.05 cd	0.13 ± 0.03 de	0.26 ± 0.04 e	0.26 ± 0.10 cdef	0.14 ± 0.01 cd	0.03 ± 0.00 abcd	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.05 ± 0.01 c	0.04 ± 0.02 bc	0.04 ± 0.00 ab
Xiaoyan 6	0.36 ± 0.09 abc	0.34 ± 0.05 ab	0.17 ± 0.01 cd	0.37 ± 0.07 bcd	0.24 ± 0.01 cdef	0.22 ± 0.02 abc	0.03 ± 0.01 abcd	0.03 ± 0.01 ab	0.02 ± 0.01 b	0.06 ± 0.01 ab	0.05 ± 0.01 ab	0.04 ± 0.00 ab
Xinong 223	0.37 ± 0.03 abc	0.27 ± 0.01 bcd	0.23 ± 0.03 abc	0.37 ± 0.02 bcd	0.28 ± 0.05 cdef	0.16 ± 0.08 bcd	0.03 ± 0.00 abcd	0.03 ± 0.00 ab	0.03 ± 0.00 a	0.06 ± 0.01 ab	0.06 ± 0.01 ab	0.03 ± 0.01 bc
Burt ert 937	0.33 ± 0.03 abc	0.33 ± 0.04 ab	0.14 ± 0.01 de	0.47 ± 0.03 a	0.32 ± 0.07 bcde	0.17 ± 0.04 abcd	0.03 ± 0.00 abcd	0.02 ± 0.01 c	0.02 ± 0.00 b	0.07 ± 0.00 a	0.05 ± 0.00 ab	0.02 ± 0.01 d
Marfed M	0.31 ± 0.01 bc	0.24 ± 0.04 d	0.15 ± 0.03 de	0.29 ± 0.03 de	0.22 ± 0.03 def	0.12 ± 0.02 d	0.03 ± 0.00 abcd	0.02 ± 0.00 c	0.02 ± 0.00 b	0.04 ± 0.01 d	0.03 ± 0.00 c	0.02 ± 0.00 d
Jinmai 47	0.31 ± 0.01 bc	0.29 ± 0.04 bcd	0.19 ± 0.02 bcd	0.46 ± 0.02 ab	0.31 ± 0.06 cdef	0.18 ± 0.02 abcd	0.03 ± 0.00 abcd	0.02 ± 0.00 c	0.02 ± 0.00 b	0.07 ± 0.00 a	0.05 ± 0.01 ab	0.04 ± 0.00 ab
Granato	0.38 ± 0.01 abc	0.37 ± 0.04 a	0.16 ± 0.00 d	0.37 ± 0.08 bcd	0.32 ± 0.05 bcde	0.27 ± 0.03 ab	0.03 ± 0.01 abcd	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.06 ± 0.01 ab	0.05 ± 0.01 ab	0.05 ± 0.01 a
Karcagi	0.17 ± 0.01 d	0.15 ± 0.04 e	0.09 ± 0.02 e	0.25 ± 0.03 e	0.16 ± 0.05 f	0.14 ± 0.04 cd	0.02 ± 0.00 d	0.02 ± 0.00 c	0.01 ± 0.00 c	0.04 ± 0.01 d	0.03 ± 0.01 c	0.03 ± 0.01 bc
Magnif M1	0.42 ± 0.06 a	0.29 ± 0.02 bcd	0.26 ± 0.04 a	0.47 ± 0.02 a	0.46 ± 0.03 a	0.19 ± 0.03 abcd	0.04 ± 0.00 a	0.03 ± 0.01 ab	0.02 ± 0.00 b	0.06 ± 0.00 ab	0.05 ± 0.00 ab	0.03 ± 0.00 bc
Castelporziano	0.35 ± 0.03 abc	0.33 ± 0.01 ab	0.23 ± 0.02 abc	0.40 ± 0.04 abc	0.34 ± 0.01 bcd	0.18 ± 0.06 abcd	0.04 ± 0.00 a	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.06 ± 0.00 ab	0.05 ± 0.01 ab	0.04 ± 0.01 ab
Durox	0.41 ± 0.08 ab	0.33 ± 0.02 ab	0.24 ± 0.03 ab	0.45 ± 0.05 ab	0.29 ± 0.12 cdef	0.21 ± 0.02 abc	0.03 ± 0.00 abcd	0.03 ± 0.01 ab	0.02 ± 0.00 b	0.07 ± 0.01 a	0.05 ± 0.01 ab	0.04 ± 0.00 ab
Icaro	0.43 ± 0.05 a	0.33 ± 0.02 ab	0.28 ± 0.05 a	0.47 ± 0.02 a	0.41 ± 0.03 ab	0.18 ± 0.04 abcd	0.03 ± 0.00 abcd	0.03 ± 0.00 ab	0.03 ± 0.00 a	0.06 ± 0.00 ab	0.06 ± 0.01 ab	0.03 ± 0.01 bc

Values represent means ± SD (standard deviations). Means with different letters within a column indicate significant differences (Duncan's test, $p \leq 0.05$).

Physiological traits of seedling leaves in PEG-6000 treatments

The relative water content (RWC) and chlorophyll content of leaves in each variety with PEG-6000 exposure was lower than that of the control group. Of all varieties, the RWC of *Rht9* decreased the least (Figure 2A), the chlorophyll content of *Rht13* decreased the least, and that of *Rht5* decreased the most (Figure 2B). PEG-6000 treatment significantly increased malondialdehyde (MDA) content in leaves, but decreased MDA content in *Rht9* (Figure 2C). With increased PEG-6000 stress, proline and soluble sugar contents in leaves also increased, especially in *Rht14* and *Rht15* (25.00% at 10% PEG-6000) and 43.61% (at 20% PEG-6000) in proline contents, with the greatest increase in soluble sugar content in *Rht12* (10.98% and 28.02%, respectively) (Figure 2D and 2F). The opposite trend occurred in soluble sugar and protein contents, reducing least (9.38% in both *Rht4* and *Rht15* in the 10% PEG-6000 treatment) and most (35.29% in *Rht2* at 20% PEG-6000) (Figure 2E). Superoxide dismutase (SOD) and peroxidase (POD) activities increased with increasing treatment concentrations, peaking in SOD activity at 146.45% (*rht*) and POD activity at 71.46% (*Rht8*) in the 10% PEG-6000 treatment; increases in SOD and POD activities were greatest at 358.55% (*Rht4*) and 156.11% (*Rht14*) in the 20% PEG-6000 treatment (Figure 2G and 2H).

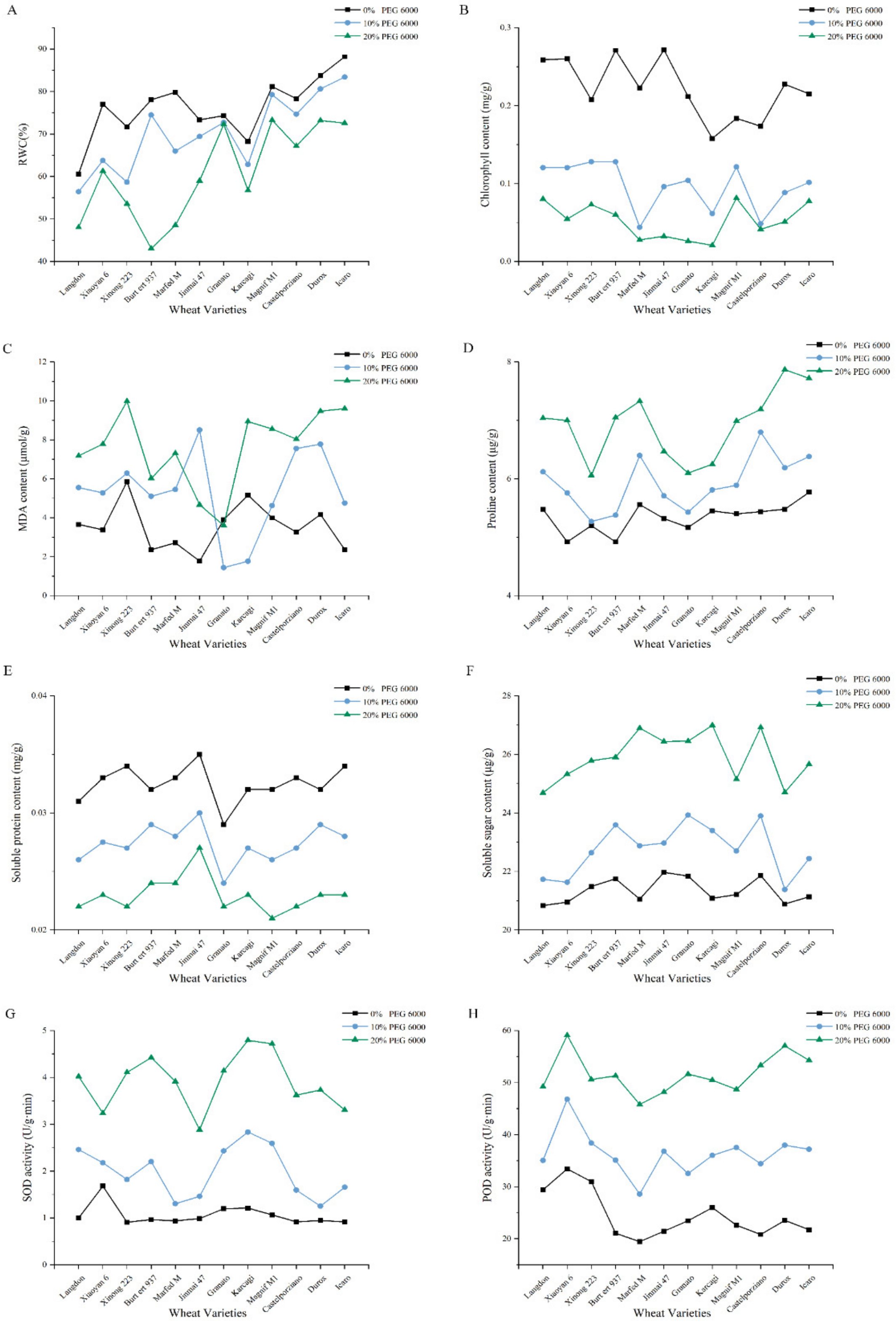
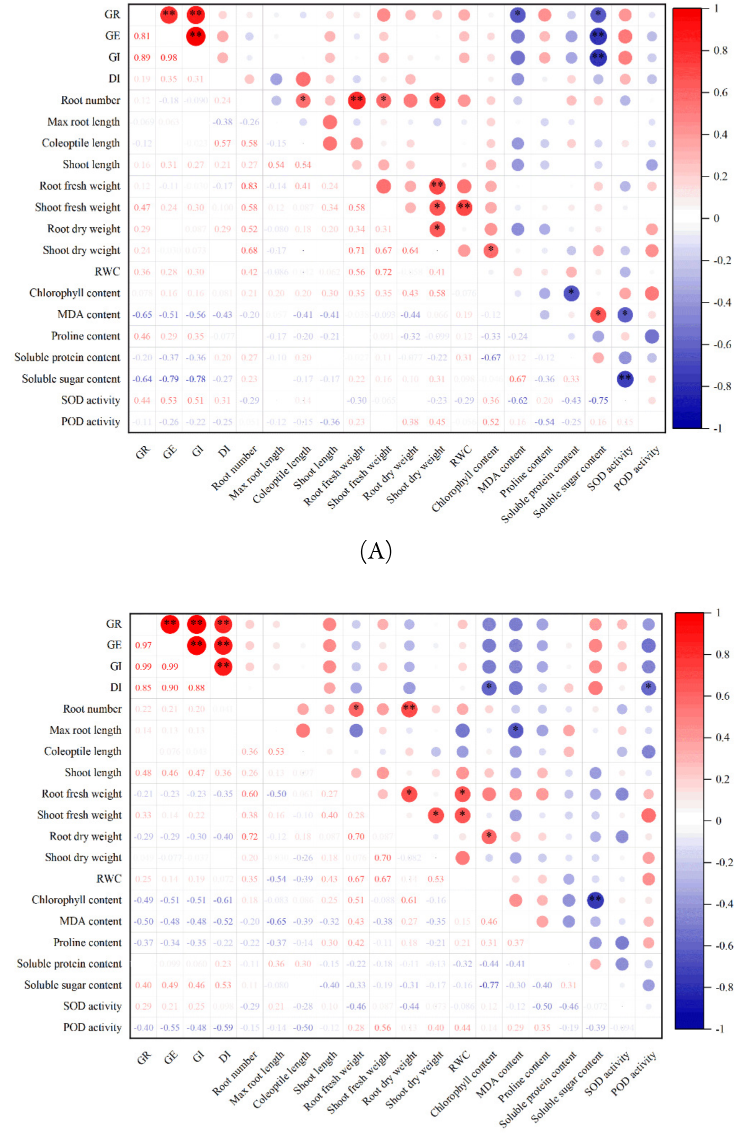


Figure 2. Effects of 10% and 20% PEG-6000 on: A) RWC; contents of B) chlorophyll, C) MDA, D) proline, E) soluble protein, F) soluble sugar; and activities of G) SOD, and H) POD for 12 wheat varieties

Correlation and principal component analyses (PCA) in PEG-6000 treatments

Significant and positive correlations occurred between GR and GE (r = 0.81), GR and GI (r = 0.89), and GE and GI (r = 0.98), root number and coleoptile length (r = 0.58), root fresh weight (r = 0.83), shoot fresh weight (r = 0.58), and shoot dry weight (r = 0.68). Relationships between SOD activity and MDA content (r = -0.75), and soluble protein content (r = -0.62) with 10% PEG-6000 treatment were significant and negative (Figure 3A). Seed germination traits were significantly and positively correlated, while a significant and negative correlation occurred between chlorophyll and soluble sugar contents (r = -0.77) in the 20% PEG-6000 treatment (Figure 3B).



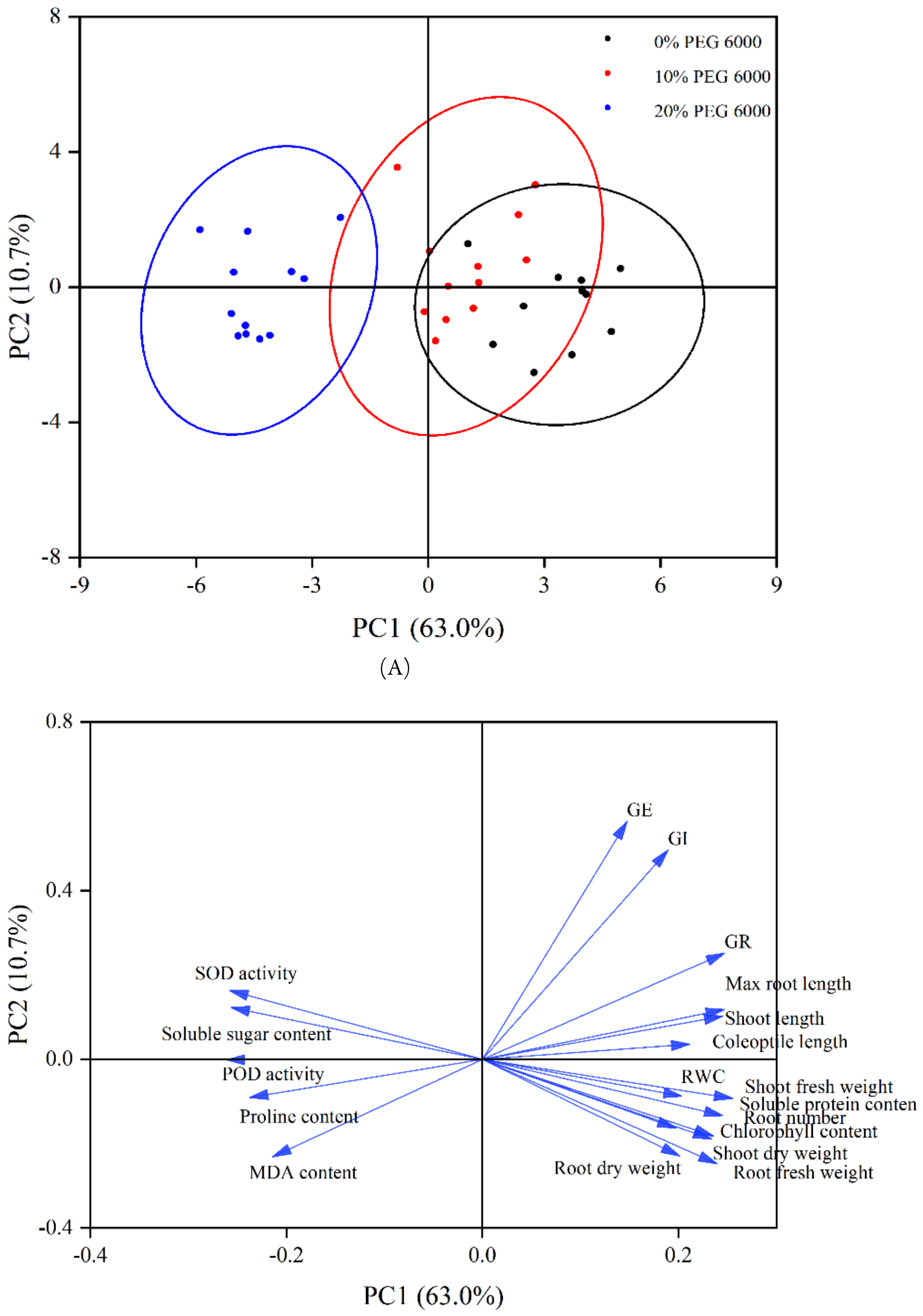
(A)

(B)

Figure 3. Correlation analysis of the investigated traits under 10% (A) and 20% (B) PEG-6000 treatments

*numbers represent correlation coefficients (r) between two indicators: +, positive correlation; -, negative correlation

The control group (0% PEG-6000) and 20% PEG-6000 were well separated while there was partial overlap between the 10% PEG-6000 and the control group in PC1, explaining 63% of the total variation (Figure 4A). Indicators with the largest positive and negative explanatory contributions under PC1 were shoot fresh weight (0.26) and POD activity (-0.26) (Figure 4B).



(A)

(B)

Figure 4. The scores plot (A) and loading plot (B) of principal component analysis (PCA) under 10% (A) and 20% (B) PEG-6000 treatments

*PC1, principal component 1; PC2, principal component 2. Values on horizontal and vertical axes indicate load factor ranges

Evaluation of drought resistance in the PEG-6000 treatment

The comprehensive drought resistance coefficient D value was used to evaluate drought resistance in each wheat variety when treated with PEG-6000; the D value correlated positively with drought resistance; D ranged 0.455–0.777 in 10% PEG-6000 treatments, and was greatest in *rht* (0.777), followed by *Rht4* (0.764) and *Rht2* (0.762) (Table 4). After 20% PEG-6000 treatment, D ranged 0.553-0.814, with the most drought resistant variety being *Rht2* (0.814) followed by *Rht9* (0.808) (Table 4).

Table 4. Comprehensive drought resistance coefficient D value for 12 wheat varieties in 10% and 20% PEG 6000 treatments

Wheat Varieties	D value (10% PEG 6000)	Rank	D value (20% PEG 6000)	Rank
Langdon	0.777	1	0.655	8
Xiaoyan 6	0.507	11	0.553	12
Xinong 223	0.762	3	0.814	1
Burt-ert 937	0.764	2	0.703	5
Marfed M	0.455	12	0.671	6
Jinmai 47	0.667	7	0.650	9
Granato	0.660	8	0.808	2
Karcagi	0.628	10	0.657	7
Magnif M1	0.707	4	0.724	4
Castelporziano	0.692	5	0.630	10
Durox	0.653	9	0.617	11
Icaro	0.676	6	0.747	3

Physiological characters and yield components under drought stress in pot experiments

The Soil and Plant Analyzer Development (SPAD) value was reduced significantly while values for other traits, including MDA content, soluble protein content, and soluble sugar, SOD, and POD activities increased significantly with drought stress; as the degree of drought increased, the response of wheat to it also intensified (Figure 5).

In moderate and severe drought conditions, reductions in plant height, spike length, and flag leaf area were apparent compared with control plants (Table 5). The three tallest plants were *rht*, *Rht13*, and *Rht14*, and the shortest was *Rht5*; the smallest decrease in plant height occurred in *Rht18* (5.54%) and *Rht12* (12.72%) (S3). The longest spikes occurred in *rht*, followed by *Rht9* at both moderate and severe drought stress, but the difference was not significant. The greatest increase in leaf area occurred in *Rht8* (at normal moisture treatment), while the smallest decrease was *Rht18* under drought stress. Reduced spikelet number spike^{-1}, grain number spike^{-1}, and 1000-kernel weight occurred in all plants with medium and severe water stress, with the maximum spikelet number spike^{-1} and grain number spike^{-1} occurring in *Rht2*, and the minimum in drought-stressed *Rht15* (Table 6; S4). The highest and lowest 1000-kernel weights occurred in water-deficit *Rht13* and *Rht15*, respectively, and the difference between them was significant.

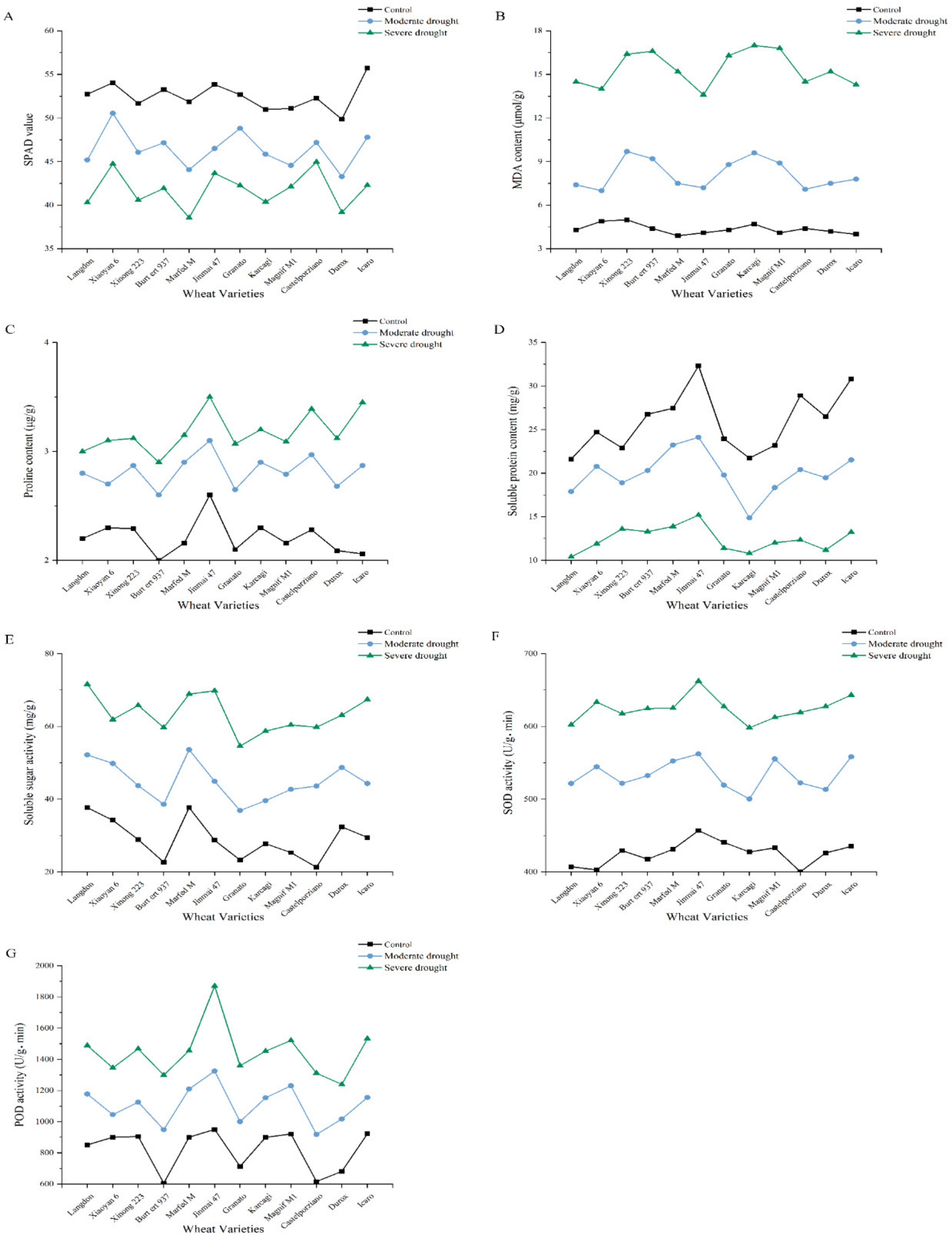


Figure 5. Effects of moderate and severe drought on: A) SPAD value; contents of B) MDA, C) proline, D) soluble protein, and E) soluble sugar; and activities of F) SOD and G) POD for 12 wheat varieties

Table 5. Plant height and flag leaf area for 12 wheat varieties under moderate and severe drought

Wheat Varieties	Plant height (cm)			Spike length (cm)			Flag leaf area (cm^2)		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Langdon	69.55 ± 1.54 a	62.93 ± 1.61 a	56.48 ± 1.90 a	8.92 ± 0.31 a	7.90 ± 0.16 a	6.87 ± 0.61 a	20.61 ± 1.36 e	15.58 ± 1.13 h	13.56 ± 0.74 e
Xiaoyan 6	60.43 ± 1.39 bc	50.92 ± 1.01 e	45.17 ± 2.63 e	8.10 ± 0.86 b	6.50 ± 0.45 c	5.73 ± 0.57 fg	24.68 ± 1.23 b	18.42 ± 1.49 d	13.76 ± 1.27 e
Xinong 223	59.42 ± 2.65 c	54.06 ± 2.92 d	49.46 ± 2.76 cd	7.90 ± 0.37 bc	7.20 ± 0.27 b	6.63 ± 0.32 b	16.87 ± 2.01 g	13.13 ± 1.37 i	9.05 ± 0.78 g
Burt ert 937	58.96 ± 2.59 c	51.33 ± 2.66 e	49.46 ± 1.26 cd	7.88 ± 0.47 bc	6.61 ± 0.50 c	5.57 ± 0.44 g	21.26 ± 1.20 d	16.10 ± 1.68 gh	12.35 ± 1.29 gh
Marfed M	58.58 ± 1.85 c	49.99 ± 1.93 e	44.53 ± 1.11 e	7.33 ± 0.46 c	6.64 ± 0.44 c	5.71 ± 0.45 fg	16.09 ± 1.15 h	12.65 ± 0.82 i	9.41 ± 0.65 g
Jinmai 47	61.09 ± 2.03 bc	54.59 ± 2.18 d	50.57 ± 1.56 bc	7.75 ± 0.25 bc	6.88 ± 0.53 bc	6.07 ± 0.47 c	25.83 ± 1.31 a	20.02 ± 1.04 b	15.99 ± 1.15 c
Granato	61.12 ± 2.21 bc	55.70 ± 1.35 cd	48.60 ± 1.43 d	8.90 ± 0.73 a	7.70 ± 0.24 a	6.83 ± 0.51 a	21.94 ± 1.24 c	17.72 ± 1.62 de	13.94 ± 1.32 e
Karcagi	57.82 ± 2.09 c	51.77 ± 1.46 e	50.47 ± 1.04 bc	7.78 ± 0.33 bc	6.59 ± 0.37 c	5.88 ± 0.70 def	21.08 ± 1.16 de	16.52 ± 1.38 fg	12.74 ± 1.16 f
Magnif M1	63.65 ± 2.59 b	58.85 ± 2.29 b	51.80 ± 1.58 b	7.83 ± 0.41 bc	6.50 ± 0.61 c	5.77 ± 0.39 def	19.23 ± 2.21 f	17.07 ± 1.85 ef	15.16 ± 1.39 d
Castelporziano	62.08 ± 1.05 bc	56.40 ± 1.68 bcd	51.07 ± 1.82 bc	8.08 ± 0.34 b	7.00 ± 0.65 bc	5.93 ± 0.42 cde	25.10 ± 2.14 b	21.19 ± 0.92 a	17.94 ± 1.44 a
Durox	60.09 ± 1.69 bc	55.32 ± 1.99 cd	49.93 ± 1.75 cd	7.81 ± 0.30 bc	6.80 ± 0.26 bc	5.83 ± 0.29 def	21.83 ± 1.17 c	19.21 ± 1.53 c	15.97 ± 1.27 c
Icaro	57.26 ± 1.92 c	54.09 ± 1.62 d	49.83 ± 1.62 cd	7.25 ± 0.25 c	6.54 ± 0.19 c	5.97 ± 0.48 cd	20.82 ± 1.43 de	19.29 ± 1.55 bc	16.89 ± 1.23 b

Table 6. Yield components of the 12 wheat varieties under moderate and severe drought

Wheat Variety	Spikelet number spike^{-1}			Grain number spike^{-1}			1000-kernel weight (g)		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Langdon	22.78 ± 2.84 ab	18.96 ± 1.22 ab	13.67 ± 1.69 ab	45.73 ± 2.84 b	36.24 ± 2.31 b	27.38 ± 1.71 cde	41.26 ± 3.41 ab	38.83 ± 2.76 ab	36.33 ± 1.14 bc
Xiaoyan 6	20.04 ± 1.41 b	17.31 ± 1.29 bc	13.31 ± 1.24 ab	41.35 ± 1.97 e	35.64 ± 2.45 b	25.67 ± 1.14 e	37.33 ± 2.16 def	36.05 ± 3.09 cd	34.53 ± 1.68 d
Xinong 223	23.98 ± 2.86 a	20.60 ± 1.20 a	14.67 ± 1.19 a	47.95 ± 2.09 a	40.11 ± 3.07 a	29.36 ± 0.97 ab	38.48 ± 1.92 cd	37.94 ± 2.69 bc	35.80 ± 1.16 c
Burt ert 937	20.04 ± 1.80 b	16.72 ± 2.13 bc	10.33 ± 0.94 b	41.46 ± 1.48 e	32.36 ± 2.96 cd	22.35 ± 1.28 f	37.96 ± 1.14 de	36.21 ± 1.92 cd	34.40 ± 1.03 d
Marfed M	22.68 ± 1.64 ab	18.73 ± 2.87 abc	12.67 ± 1.88 ab	43.68 ± 2.91 cd	37.12 ± 3.03 b	25.69 ± 1.47 e	36.32 ± 2.51 ef	35.44 ± 3.27 d	33.68 ± 1.22 d
Jinmai 47	20.01 ± 1.08 b	17.19 ± 1.44 bc	14.33 ± 1.98 a	43.97 ± 1.36 bc	36.97 ± 2.36 b	29.76 ± 1.66 a	40.08 ± 2.36 bc	39.53 ± 2.32 ab	36.93 ± 2.45 b
Granato	22.84 ± 1.47 ab	17.29 ± 2.00 bc	12.33 ± 2.03 ab	44.13 ± 1.72 bc	33.93 ± 2.51 c	26.32 ± 1.80 de	38.78 ± 2.86 cd	36.35 ± 2.39 cd	34.07 ± 1.26 d
Karcagi	20.33 ± 1.28 b	16.52 ± 2.03 bc	12.67 ± 1.27 ab	41.86 ± 2.94 de	33.07 ± 2.62 cd	26.39 ± 1.24 de	37.04 ± 2.33 def	36.48 ± 2.37 cd	33.77 ± 1.69 d
Magnif M1	22.58 ± 1.23 ab	16.44 ± 2.27 bc	13.00 ± 0.81 ab	45.62 ± 2.47 b	33.29 ± 2.58 cd	28.37 ± 2.03 bcd	41.96 ± 3.15 a	40.03 ± 2.43 a	38.37 ± 2.07 a
Castelporziano	20.82 ± 2.55 b	16.85 ± 0.83 bc	13.33 ± 0.94 ab	39.41 ± 1.86 f	32.58 ± 2.48 cd	27.67 ± 1.62 cd	37.28 ± 2.18 def	36.39 ± 2.64 cd	34.57 ± 1.38 d
Durox	21.29 ± 0.57 ab	15.91 ± 0.94 c	11.33 ± 0.99 ab	42.04 ± 3.52 de	31.73 ± 1.97 d	23.67 ± 0.98 f	36.04 ± 1.93 f	34.76 ± 1.66 d	32.17 ± 2.28 e
Icaro	20.42 ± 1.80 b	17.07 ± 2.44 bc	13.10 ± 0.85 ab	41.96 ± 3.16 de	36.46 ± 2.05 b	26.67 ± 1.55 cde	38.52 ± 2.64 cd	37.71 ± 1.73 bc	35.87 ± 1.12 c

Correlation and principal component analyses (PCA) under drought stress in pot experiments

There were highly significant positive correlations between spikelet number spike^{-1} and grain number spike^{-1} (r = 0.86 and 0.93, respectively), and soluble protein content and SOD activity (r = 0.75 and 0.72, respectively), while soluble sugar and MDA contents (r = -0.69 and -0.57, respectively) exhibited a significant negative correlation under drought stress (Figure 6A, 6B). The three groups were well separated in PC1, explaining 80.5% of the total variation (Figure 7A). The largest positive and negative explanatory contributions of all indicators under PC1 were grain number spike^{-1} (0.29) and SOD activity (-0.30) (Figure 7B).

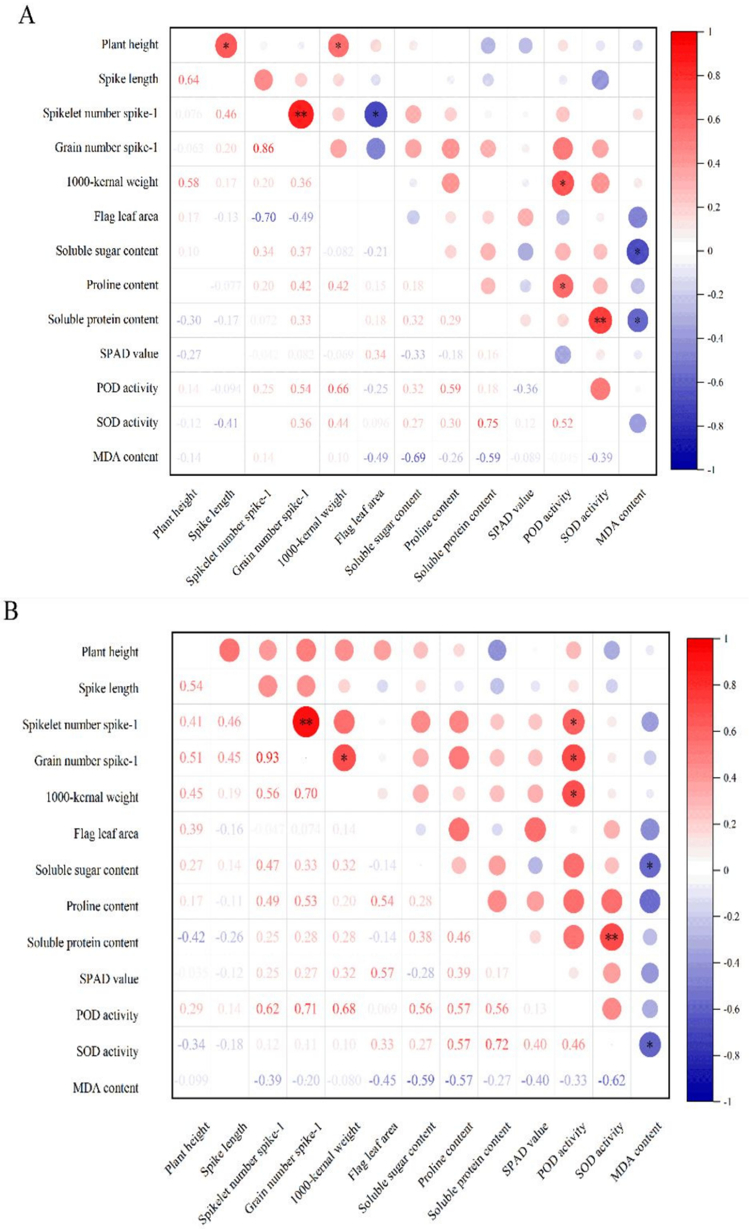


Figure 6. Correlation analysis of investigated traits under moderate (A) and severe (B) drought

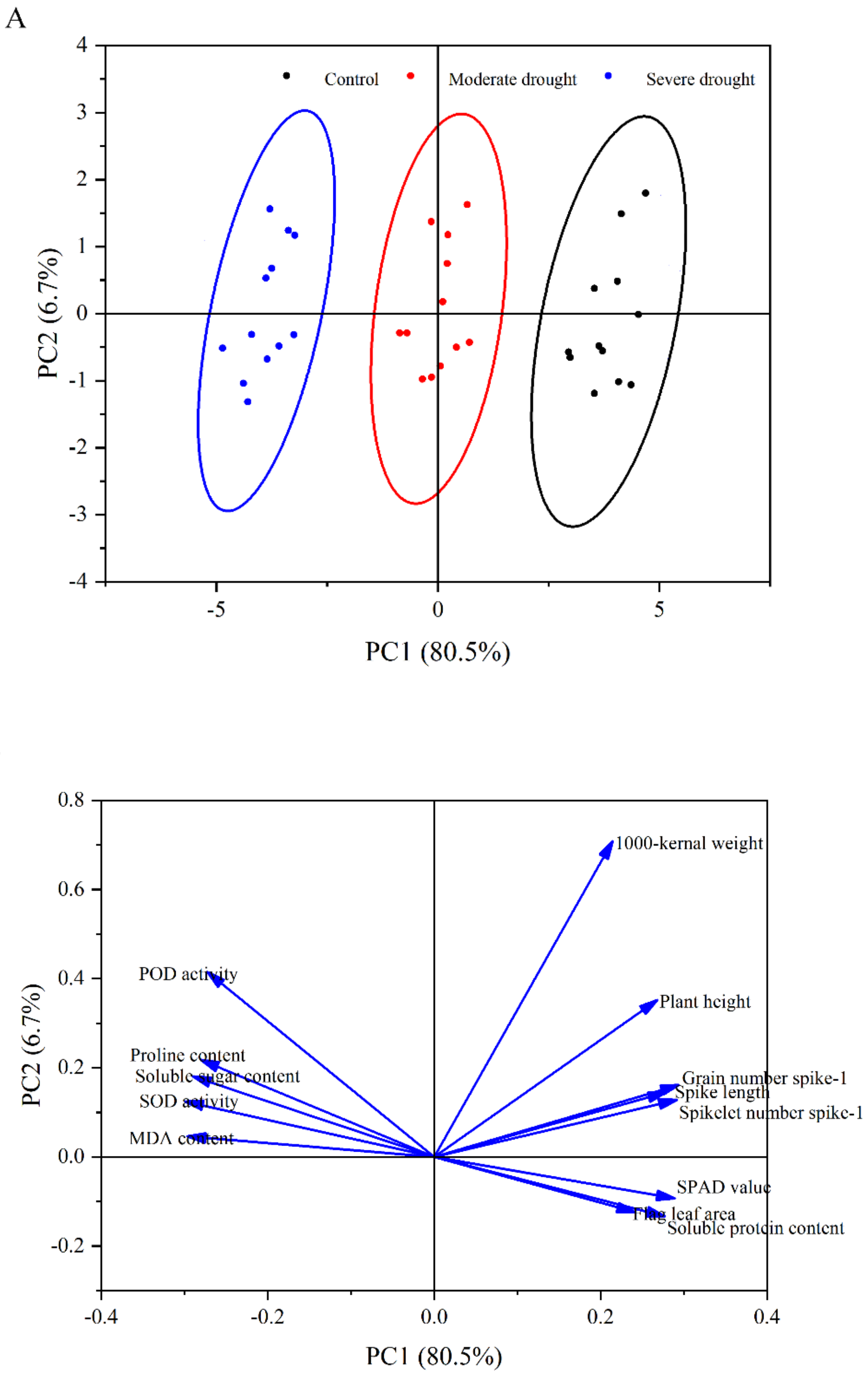


Figure 7. The scores (A) and loading (B) plots of principal component analysis (PCA) under moderate and severe drought

D values ranged 0.181 (*Rht14*) to 0.941 (*Rht8*) and 0.178 (*Rht15*) to 0.975 (*Rht8*) under moderate and severe drought stress, respectively. Based on comprehensive analysis, *Rht8* showed extremely strong drought resistance at maturity, followed by *Rht13* (Table 7).

Table 7. Comprehensive drought resistance coefficient D value for 12 wheat varieties under moderate and severe drought

Wheat Varieties	D value (Moderate drought)	Rank	D value (Severe drought)	Rank
Langdon	0.566	5	0.349	6
Xiaoyan 6	0.459	7	0.308	7
Xinong 223	0.471	6	0.369	4
Burt-ert 937	0.241	11	0.211	11
Marfed M	0.708	3	0.369	5
Jinmai 47	0.941	1	0.975	1
Granato	0.267	9	0.285	8
Karcagi	0.381	8	0.256	9
Magnif M1	0.758	2	0.408	3
Castelporziano	0.181	12	0.238	10
Durox	0.243	10	0.178	12
Icaro	0.669	4	0.546	2

Discussion

Effects of drought stress on seed germination

Water plays an important role in crop growth and development (Liu *et al.*, 2018). Identifying drought resistance in wheat seed germination is important to save agricultural water and ensure food security. Drought stress induced by polyethylene glycol (PEG) 6000 significantly decreased wheat germination, germination index, and radicle length and number (Li *et al.*, 2020c). We report seed germination to decrease significantly with increased PEG-6000 concentration, but for some differences at 10% concentration to not be significant (e.g., *Rht14* and *Rht18* in germination rate (GR); *rht*, *Rht2*, *Rht4*, *Rht5*, *Rht9*, *Rht12*, *Rht13*, *Rht15*, and *Rht18* in germination energy (GE); *rht*, *Rht2*, *Rht9*, *Rht12–15*, and *Rht18* in germination index (GI)); a similar result was reported for alfalfa (Zhang *et al.*, 2021). Wheat seeds exhibit low moisture absorption under strong drought, with the germination drought-resistant index (DI) ranging 0.22 (*Rht1*) to 0.81 (*Rht9*), possibly indicating a stronger drought-resistance in seed germination in *Rht9*.

Effects of drought stress on shoot and root growth traits

Plant growth-related traits such as the number, length, fresh and dry weight of roots, and the length, fresh, and dry weights of shoots, are typically used to screen efficient wheat varieties with water deficits (Foito *et al.*, 2009; Comas *et al.*, 2013; Janiak *et al.*, 2016). We report differences in PEG-6000 concentrations to affect wheat variety shoot and root traits in different ways (e.g., root number, and maximum root, coleoptile, and shoot lengths of some varieties were promoted by 10% PEG-6000, with relatively greater increases in *rht* and *Rht2*); similar results have been reported for wheat and *Scutellaria baicalensis* (Cheng *et al.*, 2018; Robin *et al.*, 2021). Reductions in shoot- and root-related traits occurred in each wheat variety in the 20% PEG-6000 treatment, with relatively small decreases in *Rht2*, suggesting that this variety had a greater water-use efficiency under water deficit. Promoting shoot growth is the main way to improve plant performance in drought conditions; to harmonize the promotion of root growth and improve water absorption (Claeys and Inzé, 2013), the shoot length of *Rht13* was higher (significant difference from other wheat varieties) in drought conditions, indicating that it was more drought resistant.

Effects of drought stress on leaf physiological traits

Maintaining cell moisture is essential for cell growth and development, and relative water content (RWC) and drought resistance are likely to be closely related (Grzesiak *et al.*, 2012). While we report reduced RWC in all PEG-6000-treatments, *Rht9* reduced the most. Another parameter of drought stress, chlorophyll content (Soil and Plant Analyzer Development (SPAD) value at maturity), showed a similar trend to RWC. We report malondialdehyde (MDA) to increase in leaves following PEG-6000 treatment or water shortage, excepting *Rht9* in the seedling stage, for which levels were comparable to controls, suggesting maintenance of the relative integrity of cell membranes, as reported for alfalfa (Zhang *et al.*, 2021).

Oxidative stress is a major consequence of drought. Excessive reactive oxygen species (ROS) production causes photoinhibition and oxidative damage to lipids, and disrupts enzyme activity, leading to cell death (Gill and Tuteja, 2010). We report an increase in soluble sugar and proline contents, and superoxide dismutase (SOD) and peroxidase (POD) activities, and a decrease in soluble protein content in wheat varieties under drought stress. Consequently, this may be a common strategy for wheat varieties to combat water deficit conditions. Additionally, the highest increase in proline content and SOD activity occurred in *Rht9*, in *Rht14* in POD activity, and in *Rht12* in soluble sugar content. The lowest reduction in soluble protein content occurred in *Rht8* in the highest drought stress treatment during the seedling stage, and *Rht18* in proline content and *Rht1* in SOD activity had the highest increase, and *Rht18* in soluble protein content reduced the least at maturity. This implies that these varieties might be capable of maintaining membrane stability. Cheng *et al.* (2015) reported a decrease in soluble protein content but an increase in proline content in wheat subjected to drought stress at different growth stages. Our results are consistent with those of Abid *et al.* (2018), who reported wheat to upregulate the scavenging of ROS by enhancing antioxidant enzyme activity.

Correlation and principal component (PCA) analyses

We report significant and positive coefficients of correlation for most seed-gemination traits as a consequence of drought stress. Root number significantly, and positively correlated with root fresh weight, and shoot fresh weight was positively and highly significantly correlated with shoot dry weight and RWC; a negative correlation was found between chlorophyll and soluble protein contents under drought stress. Positive, highly significant correlations were reported for shoot fresh and dry weights and RWC (Khan *et al.*, 2013; Ahmed *et al.*, 2022). Understanding the relationships between these traits is important for improving the efficiency of wheat drought-resistance breeding.

Our PCA results reveal a clear separation between the 20% PEG-6000 and control treatments, but the separation between the 10% PEG-6000 group and the other two groups is imperfect; plants in control, moderate and severe drought stress groups separated well. Significant differences existed between the 20% PEG-6000 treatment and control and the three groups in the adult stage. Of the four main biplot axes, the upper right axis indicates a positive impact on PC1 and PC2 (i.e., seed germination traits and root, shoot, and coleoptile lengths, plant height and yield components; and an increase in most parameters, except antioxidant defense-system-related indicators). However, many traits (i.e., flag leaf area, SPAD value) showed a negative impact on PC2, as reported by Marček *et al.* (2021) and Ahmed *et al.* (2022).

Before performing correlation and PCA, variance inflation factor (VIF) analysis was used to assess multicollinearity between variables. Results indicate that GR, GE, GI, shoot fresh weight, chlorophyll, soluble protein, and soluble sugar contents, and SOD and POD activities were strongly collinear in the seedling stage, and that plant height, spikelet number spike^{-1}, grain number spike^{-1}, SPAD, MDA content, and SOD and POD activities were highly collinear at maturity. This indicates a close synergistic relationship between the physiological processes represented by these indicators, and that they may have similar response patterns under drought stress. These findings suggest that only one or a few indicators need to be measured to evaluate drought resistance when a set of indicators are strongly correlated or collinear.

D value of wheat varieties

Changes in various indicators during the seedling stage when subjected to drought stress differed, and the most drought-resistant variety varied for each indicator. Thus, a comprehensive evaluation of the 19 seedling indicators and 13 adult indicators by subordinate function values analysis determined the best indicator of drought resistance during the seedling stage was based on a combination of all indicators. According to D values, the drought resistance of varieties differed depending on the drought level. Following exposure to 10% PEG-6000, the tall variety (*rht*) was most drought resistant, while dwarf varieties were more resistant at a greater level of drought stress. At maturity, *Rht8* was the most drought resistant. This indicates that some dwarf genes, like *Rht8*, *Rht13*, and *Rht2*, may be more appropriate to grow in arid and semi-arid areas. These results are consistent with findings that dwarf genes perform better under drought stress compared with the tall wild type (Kocheva *et al.*, 2014; Alghabari *et al.*, 2016).

Effects of drought stress on plant height and yield

Reductions in plant height and yield in wheat varieties under drought stress have been previously reported (Foito *et al.*, 2009). We report the least reduction in plant height and spikelet number and grain number spike^{-1} to occur in *Rht18*, and in 1000-kernel weight to occur in *Rht8*, suggesting that *Rht8* may be most resistant to drought (according to D values). Drought resistance differed in seedling and mature stages.

To better understand how wheat varieties cope with long-term water deficit, we intend to extend our research to examine wheat short-term drought time to the three leaf stage, and the long-term (from jointing stage to maturity stage) drought effects on wheat.

Conclusions

We report seed-germination (germination rate (GR), germination energy (GE) and germination index (GI), growth, and physiological traits of shoots, roots, and flag leaves, plant height and yield components in 12 wheat varieties to be more greatly affected with an increased degree of drought. The most drought-resistant wheat varieties were Langdon (*rht*) at 10% PEG, Xinong 223 (*Rht2*) at 20% PEG-6000, and Jinmai 47 (*Rht8*) at maturity. Under drought conditions in pot experiments, plant height, flag leaf area, and yield were reduced in each wheat variety. To better understand the drought tolerance of these dwarf genes, field tests are required to complement those of our pot experiments.

Based on our findings, we suggest that Xinong 223 (*Rht2*) and Jinmai 47 (*Rht8*) should be planted in drought-prone or otherwise water-deficient areas. In 2023-2024, these two wheat varieties have shown strong drought resistance in arid regions that depend on natural precipitation. Both varieties will be promoted to farmers after further trials. By selecting and cultivating drought-resistant wheat varieties, farmers can increase yields with reduced water input, thereby improving food security and economic stability. The potential economic benefits of adopting drought-resistant varieties are substantial, particularly for smallholder farmers in water-scarce regions.

Authors' Contributions

Data curation: PA; Formal analysis: XZ; Investigation: XL, JZ, XW, LX; Methodology: JZ; Software: SD; Writing - original draft: SD; Writing - review and editing: SD, PA, XZ, JZ.

All authors have read and approved the final manuscript.

Acknowledgments

The authors thank Professor Yingang Hu, College of Agronomy, Northwest A&F University, for his assistance with experimentation.

This study was supported by the Study on the Mechanism of Drought Resistance of Wheat Dwarf Genes of Longdong University [grant number XYBYZK2205]; and the Natural Science Foundation of Gansu Province [grant number 21JR7RM189].

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

References

Abid M, Ali S, Qi LK, Zahoor R, Tian Z, Jiang D, … Dai T (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Scientific Reports 8(1):4615. *https://doi.org/10.1038/s41598-018-21441-7*

Ahmed HGM, Zeng Y, Shah AN, Yar MM, Ullah A, Ali M (2022). Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. Frontiers In Plant Science 13:961049. *https://doi.org/10.3389/fpls.2022.961049*

Alghabari F, Ihsan MZ, Khaliq A, Hussain S, Daur I, Fahad S, Nasim W (2016). Gibberellin-sensitive Rht alleles confer tolerance to heat and drought stresses in wheat at booting stage. Journal of Cereal Science 70:72-78. *https://doi.org/10.1016/j.jcs.2016.05.016*

Bates LS, Waldren RPA, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207. *https://doi.org/10.1007/BF00018060*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2):248-254. *https://doi.org/10.1016/0003-2697(76)90527-3*

Buysse JAN, Merckx R (1993). An improved colorimetric method to quantify sugar content of plant tissue. Journal of Experimental Botany 44(10):1627-1629. *https://doi.org/10.1093/jxb/44.10.1627*

Cheng L, Han M, Yang L, Li Y, Sun Z, Zhang T (2018). Changes in the physiological characteristics and baicalin biosynthesis metabolism of Scutellaria baicalensis Georgi under drought stress. Industrial Crops and Products 122:473-482. *https://doi.org/10.1016/j.indcrop.2018.06.030*

Cheng Z, Dong K, Ge P, Bian Y, Dong L, Deng X, … Yan Y (2015). Identification of leaf proteins differentially accumulated between wheat cultivars distinct in their levels of drought tolerance. PloS ONE 10(5):e0125302.*https://doi.org/10.1371/journal.pone.0125302*

Claeys H, Inzé D (2013). The agony of choice: How plants balance growth and survival under water-limiting conditions. Plant Physiology 162(4):1768-1779. *https://doi.org/10.1104/pp.113.220921*

Comas LH, Becker SR, Cruz VM, Byrne PF, Dierig DA (2013). Root traits contributing to plant productivity under drought. Frontiers In Plant Science 4:442. *https://doi.org/10.3389/fpls.2013.00442*

Daryanto S, Wang L, Jacinthe PA (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. Agricultural Water Management 179:18-33. *https://doi.org/10.1016/j.agwat.2016.04.022*

Duan S, Zhao Z, Qiao Y, Cui C, Morgunov A, Condon AG, … Hu Y-G (2020). GAR dwarf gene Rht14 reduced plant height and affected agronomic traits in durum wheat (*Triticum durum*). Field Crops Research 248:107721. *https://doi.org/10.1016/j.fcr.2020.107721*

Ellis MH, Rebetzke GJ, Chandler P, Bonnett D, Spielmeyer W, Richards RA (2004). The effect of different height reducing genes on the early growth of wheat. Functional Plant Biology 31(6):583-589. *https://doi.org/10.1071/fp03207*

Farooq M, Hussain M, Siddique KHM (2014). Drought stress in wheat during flowering and grain-filling periods. Critical

Reviews in Plant Sciences 33(4):331-349. *https://doi.org/10.1080/07352689.2014.875291*

Foito A, Byrne SL, Shepherd T, Stewart D, Barth S (2009). Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG-induced water stress. Plant Biotechnology Journal 7(8):719-732. *https://doi.org/10.1111/j.1467-7652.2009.00437.x*

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48(12):909-930. *https://doi.org/10.1016/j.plaphy.2010.08.016*

Grzesiak MT, Marcińska I, Janowiak F, Rzepka A, Hura T (2012). The relationship between seedling growth and grain yield under drought conditions in maize and triticale genotypes. Acta Physiologiae Plantarum 34:1757-1764. *https://doi.org/10.1007/s11738-012-1039-2*

Hedden P (2003). The genes of the green revolution. Trends in Genetics 19(1):5-9. *https://doi.org/10.1016/s0168-9525(02)00009-4*

Ihsan MZ, El-Nakhlawy FS, Ismail SM, Fahad S, Daur I (2016). Wheat phenological development and growth studies as affected by drought and late season high temperature stress under arid environment. Frontiers in Plant Science. 7:795. *https://doi.org/10.3389/fpls.2016.00795*

Janiak A, Kwaśniewski M, Szarejko I (2016). Gene expression regulation in roots under drought. Journal of Experimental Botany 67(4):1003-1014. *https://doi.org/10.1093/jxb/erv512*

Khan MH, Bukhari A, Dar ZA, Rizvi SM (2013). Status and strategies in breeding for rust resistance in wheat. Agricultural Sciences 4(6):292. *https://doi.org/10.4236/as.2013.46042*

Kocheva K, Nenova V, Karceva T, Petrov P, Georgiev GI, Börner A, Landjeva S (2014). Changes in water status, membrane stability and antioxidant capacity of wheat seedlings carrying different *Rht-B1* dwarfing alleles under drought stress. Journal of Agronomy and Crop Science 200(2):83-91. *https://doi.org/10.1111/jac.12047*

Lei S, Zeng B, Yuan Z, Su X (2014). Changes in carbohydrate content and membrane stability of two ecotypes of *Calamagrostis arundinacea* growing at different elevations in the drawdown zone of the Three Gorges Reservoir. PLoS one 9(3):e91394. *https://doi.org/10.1371/journal.pone.0091394*

Li D, Batchelor WD, Zhang D, Miao H, Li H, Song S, Li R (2020b). Analysis of melatonin regulation of germination and antioxidant metabolism in different wheat cultivars under polyethylene glycol stress PLoS ONE 15(8):e0237536. *https://doi.org/10.1371/journal.pone.0237536*

Li D, Li C, Sun H, Wang W, Liu L, Zhang Y (2010). Effects of drought on soluble protein content and protective enzyme system in cotton leaves. Frontiers of Agriculture in China 4:56-62. *https://doi.org/10.1007/s11703-010-0102-2*

Li MQ, Yang J, Wang X, Li DX, Zhang CB, Tian ZH, … Lin HH (2020c). Transcriptome profiles identify the common responsive genes to drought stress in two *Elymus species*. Journal of Plant Physiology 250:153183. *https://doi.org/10.1016/j.jplph.2020.153183*

Li Y, Zhang L, Yu Y, Zeng H, Deng L, Zhu L, … Wang Y (2022). Melatonin-induced resilience strategies against the damaging impacts of drought stress in rice. Agronomy 12(4):813. *https://doi.org/10.3390/agronomy12040813*

Liu M, Wang Z, Xiao HM, Yang Y (2018). Characterization of *TaDREB1* in wheat genotypes with different seed germination under osmotic stress. Hereditas 155:26. *https://doi.org/10.1186/s41065-018-0064-6*

Liu R, Wu F, Yi X, Lin Y, Wang Z, Liu S, … Liu Y (2020). Quantitative trait loci analysis for root traits in synthetic hexaploid wheat under drought stress conditions. Journal of Integrative Agriculture 19(8):1947-1960. *https://doi.org/10.1016/S2095-3119(19)62825-X*

Liu Y, Zhang J, Hu Y, Chen J (2017). Dwarfing genes *Rht4* and *Rht-B1b* affect plant height and key agronomic traits in common wheat under two water regimes. Field Crops Research 204(15):242-248. *https://doi.org/10.1016/j.fcr.2017.01.020*

Mahpara S, Zainab A, Ullah R, Kausar S, Bilal M, Latif I, … Zuan K (2022). The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (*Triticum aestivum* L.) genotypes. PLoS ONE 17(2):e0262937. *https://doi.org/10.1371/journal.pone.0262937*

Marček T, Kovač T, Jukić K, Lončarić A, Ižaković M (2021). Application of high voltage electrical discharge treatment to improve wheat germination and early growth under drought and salinity conditions. Plants 10(10):2137. *https://doi.org/10.3390/plants10102137*

Mohi-Ud-Din M, Hossain MA, Rohman MM, Uddin MN, Haque MS, Ahmed JU, … Mostofa MG (2021). Multivariate analysis of morpho-physiological traits reveals differential drought tolerance potential of bread wheat genotypes at the seedling stage. Plants 10(5):879. *https://doi.org/10.3390/plants10050879*

Møller IM, Jensen PE, Hansson A (2007). Oxidative modifications to cellular components in plants. Annual Review of Plant Biology 58:459-481. *https://doi.org/10.1146/annurev.arplant.58.032806.103946*

Nagata M, Yamashita I (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaishi 39(10):925-928. *https://doi.org/10.3136/nskkk1962.39.925*

Park D, Jang J, Jang G (2025). Bacillus megaterium GEB3 reduces accumulation of reactive oxygen species and enhances drought tolerance in peppers. Applied Biological Chemistry 68: 15. *https://doi.org/10.1186/s13765-025-00989-9*

Pour-Aboughadareh A, Etminan A, Abdelrahman M, Siddique KHM, Tran L-SP (2020). Assessment of biochemical and physiological parameters of durum wheat genotypes at the seedling stage during polyethylene glycol-induced water stress. Plant Growth Regulation 92(1):81-93. *https://doi.org/10.1007/s10725-020-00621-4*

Radford BJ (1987). Effect of constant and fluctuating temperature regimes and seed source on the coleoptile length of tall and semidwarf wheats. Australian Journal of Experimental Agriculture 27(1):113-117. *https://doi.org/10.1071/ea9870113*

Rebetzke G, Bonnett D, Ellis M (2012). Combining gibberellic acid-sensitive and insensitive dwarfing genes in breeding of higher-yielding, sesqui-dwarf wheats. Field Crops Research 127:17-25. *https://doi.org/10.1016/j.fcr.2011.11.003*

Rebetzke GJ, Richards RA (2000). Gibberellic acid-sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Australian Journal of Agricultural Research 51(2):235-246. *https://doi.org/10.1071/AR99043*

Robin A, Ghosh S, Shahed M (2021). PEG-Induced osmotic stress alters root morphology and root hair traits in wheat genotypes. Plants 10(6):1042. *https://doi.org/10.3390/plants10061042*

Saddiq M, Wang X, Iqbal S, Hafeez M, Khan S, Raza A, Gulshan B (2021). Effect of water stress on grain yield and physiological characters of quinoa genotypes. Agronomy 11(10):1934. *https://doi.org/10.3390/agronomy11101934*

Sallam A, Alqudah AM, Dawood MFA, Baenziger PS, Börner A (2019). Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. International Journal of Molecular Sciences 20(13):3137. *https://doi.org/10.3390/ijms20133137*

Wang Y, Du Y, Yang Z, Chen L, Condon AG, Hu Y-G (2015). Comparing the effects of GA-responsive dwarfing genes *Rht13* and *Rht8* on plant height and some agronomic traits in common wheat. Field Crops Research 179:35-43. *https://doi.org/10.1016/j.fcr.2015.04.010*

Xu C, Xu Z, Li Y, Luo Y, Wang K, Guo L, Hao C (2024). Drought characteristics and causes during winter wheat growth stages in north China. Sustainability 16(14): 5958. *https://doi.org/10.3390/su16145958*

Zhang D, Wang G, Zhou H (2011). Assessment on agricultural drought risk based on variable fuzzy sets model. Chinese Geographical Science 21:167-175. *https://doi.org/10.1007/s11769-011-0456-2*

Zhang X, Yang S, Zhou Y, He Z, Xia X (2006). Distribution of the *Rht-B1b*, *Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers. Euphytica 152(1):109-116. *https://doi.org/10.1007/s10681-006-9184-6*

Zhang XY, Shi SL, Li XL, Li CN, Zhang CM, Kang WJ, Yin GL (2021). Effects of autotoxicity on alfalfa (*Medicago sativa*): Seed germination, oxidative damage and lipid peroxidation of seedlings. Agronomy 11(6):1027. *https://doi.org/10.3390/agronomy11061027*

Supplemental Tables

Table S1. Ratios of increase (+ve numbers) or decrease (−ve numbers) for 10% and 20% PEG 6000 treatments on root number, maximum root length, coleoptile length, and shoot length for 12 wheat varieties

Wheat Varieties	**Root number**		**Max root length (cm)**		**Coleoptile length (cm)**		**Shoot length (cm)**	
	Effect (%) (10% PEG 6000 vs. 0% PEG 6000)	**Effect (%) (20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%) (20% PEG 6000 vs. 0% PEG 6000)**
Langdon	8.89	−28.89	20.90	−56.48	32.45	−48.81	29.97	−16.58
Xiaoyan 6	−4.00	−32.00	−10.46	−62.19	−1.06	−56.73	−16.19	−39.26
Xinong 223	14.63	−7.32	14.69	−64.57	20.49	−3.28	38.29	−18.19
Burt ert 937	10.42	−20.93	−0.98	−59.41	39.15	−7.47	−1.91	−33.90
Marfed M	−6.67	−15.56	3.49	−72.04	5.12	−30.12	2.19	−34.84
Jinmai 47	−6.38	−10.64	4.90	−50.64	−6.75	−18.97	−6.45	−28.79
Granato	−20.90	−28.36	−4.25	−55.54	12.56	−51.26	10.87	−22.30
Karcagi	−5.56	−33.33	−11.30	−73.76	0.00	−57.58	−17.94	−40.49
Magnif M1	−8.16	−14.29	7.49	−73.59	7.59	−24.09	−16.80	−37.84
Castelporziano	−1.75	−26.32	10.18	−73.36	24.01	−34.87	3.19	−27.96
Durox	−4.00	−36.00	2.01	−71.64	3.67	−49.61	−14.01	−38.23
Icaro	−11.29	−29.03	−3.81	−80.46	−1.00	−66.08	−14.84	−39.24

Effect (%) = (10% PEG 6000 or 20% PEG 6000 - 0% PEG 6000)/ 0% PEG 6000 × 100%

Table S2. Ratios of increase (+ve numbers) or decrease (−ve numbers) for 10% and 20% PEG 6000 treatments on fresh weight of roots and shoots, and dry weights of roots and shoots for 12 wheat varieties

Wheat Varieties	**Root fresh weight (g)**		**Shoot fresh weight (g)**		**Root dry weight (g)**		**Shoot dry weight (g)**	
	Effect (%) **(10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(20% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(10% PEG 6000 vs. 0% PEG 6000)**	**Effect (%)** **(20% PEG 6000 vs. 0% PEG 6000)**
Langdon	−26.47	−61.76	0.00	−46.15	0.00	−33.33	−20.00	−20.00
Xiaoyan 6	−5.56	−52.78	−35.14	−40.54	0.00	−33.33	−16.67	−33.33
Xinong 223	−27.03	−37.84	−24.32	−56.76	0.00	0.00	0.00	−50.00
Burt ert 937	0.00	−57.58	−31.91	−63.83	−33.33	−33.33	−28.57	−71.43
Marfed M	−22.58	−51.61	−24.14	−58.62	−33.33	−33.33	−25.00	−50.00
Jinmai 47	−6.45	−38.71	−32.61	−60.87	−33.33	−33.33	−28.57	−42.86
Granato	−2.63	−57.89	−13.51	−27.03	0.00	−33.33	−16.67	−16.67
Karcagi	−11.76	−47.06	−36.00	−44.00	0.00	−50.00	−25.00	−25.00
Magnif M1	−30.95	−38.10	−2.13	−59.57	−25.00	−50.00	−16.67	−50.00
Castelporziano	−5.71	−34.29	−15.00	−55.00	−25.00	−50.00	−16.67	−33.33
Durox	−19.51	−41.46	−35.56	−53.33	0.00	−33.33	−28.57	−42.86
Icaro	−23.26	−34.88	−12.77	−61.70	0.00	0.00	0.00	−50.00

Effect (%) = (10% PEG 6000 or 20% PEG 6000 - 0% PEG 6000)/ 0% PEG 6000 × 100%.

Table S3. Ratios of decrease for moderate and severe drought on plant height, spike length, and flag leaf area for 12 wheat varieties

Wheat Varieties	Plant height (cm)		Spike length (cm)		Flag leaf area (cm^2)	
	Decreased radio at moderate drought (%)	Decreased radio at severe drought (%)	Decreased radio at moderate drought (%)	Decreased radio at severe drought (%)	Decreased radio at moderate drought (%)	Decreased radio at severe drought (%)
Langdon	9.52	18.79	11.43	23.02	24.41	34.20
Xiaoyan 6	15.74	25.26	19.75	29.22	25.38	44.25
Xinong 223	9.02	16.76	8.86	16.03	22.16	46.36
Burt ert 937	12.94	23.45	16.12	29.36	24.25	41.90
Marfed M	14.66	23.98	9.41	21.78	21.36	41.50
Jinmai 47	10.64	17.23	11.23	21.72	22.50	38.08
Granato	8.87	20.48	13.48	23.22	19.22	36.47
Karcagi	10.46	12.72	15.30	24.59	21.65	39.57
Magnif M1	7.54	18.62	16.99	26.35	11.24	21.16
Castelporziano	9.15	17.74	13.37	26.57	15.59	28.54
Durox	7.94	16.90	12.93	25.31	12.02	26.83
Icaro	5.54	12.97	9.79	17.70	7.33	18.88

Table S4. The decreased ratio of moderate and severe drought on yield components of the 12 wheat varieties

Wheat Varieties	**Spikelet number spike^{-1}**		**Grain number spike^{-1}**		**1000-kernel weight (g)**	
	Decreased radio at moderate drought (%)	**Decreased radio at severe drought (%)**	**Decreased radio at moderate drought (%)**	**Decreased radio at severe drought (%)**	**Decreased radio at moderate drought (%)**	**Decreased radio at severe drought (%)**
Langdon	16.77	40.01	20.75	40.23	5.89	11.94
Xiaoyan 6	13.62	33.47	13.81	37.93	3.43	7.49
Xinong 223	14.10	38.84	16.35	38.83	1.40	6.96
Burt ert 937	16.57	48.44	21.95	46.13	4.61	9.38
Marfed M	17.42	44.15	15.02	41.24	2.42	7.03
Jinmai 47	14.09	28.37	15.92	32.53	1.37	7.85
Granato	24.30	46.00	23.11	40.33	6.27	12.15
Karcagi	18.74	37.69	21.00	37.09	1.51	8.84
Magnif M1	27.19	42.43	27.03	37.89	4.60	8.56
Castelporziano	19.07	35.96	17.33	29.80	2.39	7.28
Durox	25.27	46.77	24.52	43.70	3.55	10.75
Icaro	16.41	36.34	13.11	36.45	2.10	6.89