Research article

Drought and rewatering practices improve adaptability of seedling maize to drought stress by a super-compensate effect

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ARTICLE INFO

ABSTRACT

Periodic drought adversely affects the growth and yield of summer crops in the Huang-Huai-Hai Plain. Drought-rewatering practice as one of the important agronomic measures to improve crop drought resistance. A field experiment was conducted to investigate practice physiological, biochemical, and molecular responses of maize seedling after two rounds of repeated drought and rewatering treatments. The results demonstrated that rewatering following repeated drought events had a compensatory effect on the photosynthetic rate (Pn) and on osmotic and antioxidant regulation. Specifically, the Pn and stomatal conductance (Gs) increased by 10.12 % and 5.61 %, respectively, compared to the control (CK) during the second round of treatment. Additionally, soluble protein (sPro) and proline (Pro) content rose significantly, with increases of 26.12 % and 343.49 % observed on day 5 of the second round, leading to a gradual reduction in leaf water content and osmosis. Following drought exposure, the activities of superoxide dismutase (SOD) and peroxidase (POD) contributed to the decreased levels of malondialdehyde (MDA), with both enzymes recovering during rewatering. In contrast, plant height, leaf area, and biomass were significantly reduced in the CK group. Notably, root length increased by 21.05 % after the drought-rewatering practice, enhancing the maize seedlings' ability to adapt to drought stress. Overall, maize seedlings exhibited enhanced adaptability to drought conditions following two cycles of drought-rewatering treatments.

1. Introduction

As the global climate changes, the frequency and intensity of drought events are increasing annually, particularly in arid and semi-arid regions [1,2]. However, seasonal droughts also occur in non-arid areas, posing significant challenges to agricultural productivity. Drought has emerged as a primary factor constraining crop growth and development [3,4]. These seasonal droughts threaten crop growth, affect yield and quality, and jeopardize food security [5,6]. In the Huang-Huai-Hai Plain of China, seasonal droughts frequently arise due to a severe mismatch between precipitation and the water requirements of crops, particularly during the seedling stage of summer maize (*Zea mays* L.) [7,8]. This mismatch significantly impacts the growth of maize seedlings and the eventual yield formation. Therefore, investigating the effects of periodic drought on maize and its recovery mechanisms is of great significance.

To elucidate the mechanisms by which drought influences maize growth, studies have indicated that photosynthesis is a physiological process that exhibits a pronounced response to drought [9]. For instance, our previous research observed a sharp increase in the content of reducing sugars and proline, accompanied by a significant reduction in the rates of photosynthesis and transpiration during maize seedling adaptation to drought [10]. Drought stress studies have shown that water-stressed plants exhibit significantly higher activity levels of peroxidase and catalase, along with elevated proline content, compared to well-watered plants; these levels decrease upon rewatering and demonstrate an inverse relationship with glutathione reductase activity [11].

Research has primarily focused on plant performance under long-term drought stress, encompassing aspects such as plant morphology and the molecular response mechanisms in water-deficient environments [12,13]. Studies have also demonstrated that plants exhibit memory behaviors following previous drought stress, enabling them to achieve more robust drought tolerance upon re-exposure [14,15]. This is attributed to crops generating positive outcomes related to compensation or super-compensation in physiological and biochemical metabolism, thereby mitigating damage and losses during drought stress [16,17]. Leveraging the super-compensatory responses of plants can enhance their inherent drought tolerance and reduce the yield losses caused by adverse conditions [18,19]. Many researchers have examined this phenomenon, finding that the ultimate yield of crops in arid environments is influenced not only by their ability to withstand and endure drought stress but also by their capacity to resume normal growth following rehydration [20–22]. Consequently, understanding the mechanisms underlying normal growth resumption after water supply is fundamental for enabling crops to adapt to arid environments and cope with drought stress. Studies have indicated that rewatering seedlings for 24–48 h can restore growth, rapidly increase water potential (ψ_{wa}), enhance rates of photosynthetic gas exchange, and sharply decrease the content of soluble sugars and proline [23]. Additionally, plants undergoing drought and subsequent rehydration have been shown to exhibit higher relative water content (RWC) and proline levels, alongside more effective photosynthesis and antioxidant systems [24].

Research has also indicated that recurrent drought stress can enhance overall drought tolerance in plants. Physiological 'stress memory' may develop during pre-stress events, allowing for a more rapid response when stress recurs [25]. In some cases, plants subjected to repeated cycles of drought and irrigation may recover and outperform those that have not experienced drought stress. For example, perennial grasses (Leymus chinensis) subjected to repeated moderate and severe drought conditions exhibited similar or greater tiller numbers and biomass than well-watered control treatments [26]. We hypothesize that multiple cycles of drought-rehydration can improve plant drought resistance, potentially leading to enhanced physiological traits. However, limited research has been conducted on whether the characteristics of enhanced drought tolerance following drought rehydration persist after multiple rounds of treatment, and how the corresponding physiological indicators change remains to be clarified.

In light of the aforementioned factors, this experiment was conducted to investigate the physiological and morphological responses of maize seedlings subjected to two rounds of drought-rewatering practices. The study aimed to determine whether maize seedlings exhibit compensation or super-compensation effects in response to fluctuating low water conditions. The findings are expected to inform the adjustment of cultivation practices to enhance drought adaptability, optimize the utilization of high-quality germplasm resources, and maximize crop production potential. Ultimately, this research will provide theoretical guidance for establishing a foundation for water-saving cultivation strategies in agricultural practices.

2. Materials and methods

2.1. Site description

The plot experiment was conducted in a rainfall shelter at the experimental station of Henan Agricultural University (Zhengzhou, China; 34°80′ N, 113°65′ E, 70 m above sea level) from April to July in 2017. Over the past three decades, the annual average minimum and maximum air temperatures were recorded at 10.1 °C and 20.5 °C, respectively, with a mean annual precipitation of 632 mm (ranging from 380 to 991 mm). The soil texture at the experimental site was sandy loam, with a volumetric water content at field capacity 22.87 %. The basic chemical parameters of the 0–30 cm soil layer were as follows: total nitrogen 0.72 g kg^{-1}, Available phosphorus 9.8 mg kg^{-1}, Available potassium 124 mg kg^{-1}, Organic matter 9.59 g kg^{-1}, and alkaline hydrolyzed nitrogen 17 mg kg^{-1}. Soil samples were collected from the cultivated soil layer, screened, dried, and mixed with compound fertilizer as a base nutrient, consistent with farmer practices.

2.2. Experimental design and management

The experiment utilized plastic barrels of uniform specification (18 cm in diameter and 25 cm in height) with completely closed bottoms. Soil was added according to a bulk density of 1.2 g cm^{-3}, filling to 2 cm from the top of the barrels. Each pot was uniformly mixed with approximately 3.8 g of urea, following typical fertilization levels. To prevent surface water evaporation and soil compaction after watering, a PVC tube (26 cm in length, 1 cm in diameter) was vertically placed in each barrel. The tube was perforated and wrapped in fine mesh to prevent soil blockage. After saturating the soil with water, the barrels were allowed to dry naturally to 75 % FC (verified through weighing calculations) before seeding.

Maize *(Zea mays* L.*)* cultivar Zhengdan 958, a predominant summer maize variety in the Huang-Huai-Hai Plain, was selected for this study, which has an approximate growth duration of 96 days. Seeds were sown on May 1, 2017, with four seeds planted per pot, totaling 40 pots. Post-sowing, the weight of each pot was measured daily (using an electronic balance with an accuracy of 0.1 g) at 7:00 p.m. Any water loss was slowly replenished from the top of the PVC tube based on the subtraction method to maintain 75 % FC.

When maize reached the trefoil stage on May 15, 2017, two consecutive rounds of drought-rewatering treatments were

implemented for the treatment group (T). The first round involved withholding water from May 15, allowing soil moisture to decrease from 75 % FC to 25 % FC over a six-day period (2 days at 75 % FC to 40 % FC, followed by 3 days to reduce to 25 % FC). Subsequently, the soil was rewatered to 75 % FC for 3 days (R3). The second round followed the same protocol, with 8 days of treatment and 20 repetitions. The control group (CK) was maintained at 75 % FC daily without drought treatment. All treatments were subjected to identical management conditions, except for the differing water regimes. The daily relative soil water content (SWC) is illustrated in Fig. 1.

2.3. Sample collection and data calculation

Soil samples were collected from 0 to 20 cm and 20–40 cm layers using a soil drill and aluminum boxes, after which basic soil bulk density, moisture, and nutrient levels were determined. Plant height and leaf area were measured at 2 days (D2), 5 days (D5) of drought, and 3 days of rehydration (R3). Plant samples were collected concurrently.

2.3.1. Plant photosynthesis parameters

The photosynthesis rate (Pn) and stomatal conductance (Gs) of the flag leaf (the first fully expanded leaf from the top at the jointing stage) were measured using a LI-6400 Portable Photosynthesis System (LI-Cor, Inc., Lincoln, NE, USA) between 9:30 a.m. and 11:30 a. m. on each treatment day.

2.3.2. Calculation of soil field capacity and daily water supplement

Field capacity (FC) was determined by the method [27] (Eq. (1)). The soil moisture (Mt) measured after saturated water absorption, and then dried at 105 °C to constant weight, and the dry soil weight (Ms) was recorded.

$$FC = \frac{(Mt - Ms)}{Ms} \times 100\% \tag{1}$$

The daily water supply Ws (g or mL) was calculated by Eq. (2):

$$W_s = (M - M_a) \times 1000 \tag{2}$$

where M is the weight of soil FC for 75 %, Ma is actual measured weight.

The Relative soil water content (SWC) was calculated by Eq. (3):

$$SWC = \frac{W_c}{FC} \times 100\% \tag{3}$$

where Wc is daily actual water content, FC is field capacity.

2.3.3. The range of the moisture threshold

The photosynthetic rate (Pn) and the relative SWC (%) were determined daily after the treatment began. Using a linear equation to establish the Pn (y) and the relative soil
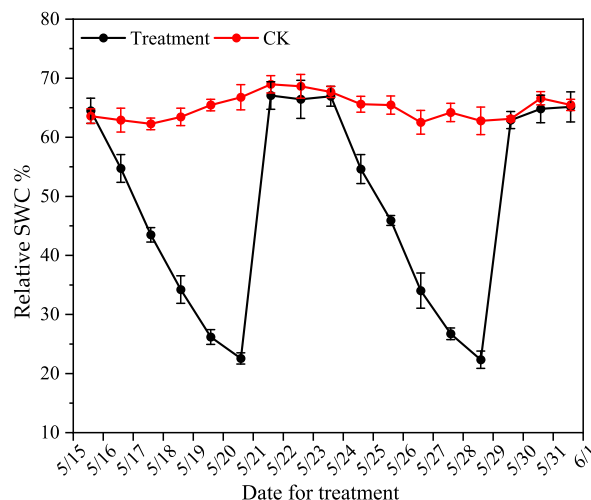water content (x). the minimum theoretical soil water content is when the Pn equals 0.

Fig. 1. Relative water content of soil in two rounds of treatment.

2.3.4. Determination antioxidant regulation indicators

The superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) method [28] (Eq. (4)). After the color reaction, the absorbance of the sample was obtained at 560 nm and calculated. One unit of SOD was defined as the amount of enzyme that inhibited the NBT reduction by 50 %, SOD activity ($U \cdot g^{-1} \cdot FW \cdot min^{-1}$)

$$SOD\left(U \cdot g^{-1} \cdot FW \cdot min^{-1}\right) = \frac{(A_0 - As) \times Vt}{A_0 \times 0.5 \times W \times Vs \times t} \tag{4}$$

where, A_0: the light absorption value of the light control tube; As: the light absorption value of the sample tube; Vt: the total volume of the sample liquid (ml); Vs: the sample amount (ml); W: fresh sample weight (g).

The Peroxidase (POD) activity was determined by the colorimetric method [29] (Eq. (5)), the change of A_{470} per minute was 0.01 as one peroxidase activity unit (U).

$$POD\left(U \cdot g^{-1} \cdot FW \cdot min^{-1}\right) = \frac{\Delta A_{470} \times Vt}{W \times Vs \times 0.01 \times t} \tag{5}$$

where ΔA_{470}: change of absorbance in the reaction time; W: the fresh weight of the plant (g); Vt: the total volume of the extracted enzyme solution (mL); Vs: the volume of the enzyme solution (mL); t: the reaction time (min).

MDA (Malondialdehyde) contents was determined by the thiobarbituric acid method (TBA) (Eq. (6) and Eq. (7)). Measured absorbance of the reaction solution at 600 nm, 532 nm, and 450 nm to calculate the MDA content:

$$C(uM) = 6.45 \times (D_{532} - D_{600}) - 0.56 D_{450} \tag{6}$$

$$MDA\left(umol \cdot g^{-1}\right) = \frac{C \times N}{W} \tag{7}$$

where C: MDA concentration, N: total volume of extract (mL), W: fresh sample weight (g).

2.3.5. Determination of osmotic adjustment indictors

Relative water content of leaves (RWC). The fresh leaves were weighed (Wf), and the leaves were immersed in distilled water for 5 h to absorb water and weighed to obtain the plant tissue water saturation weight (*Wt*). Finally, the sample was dried and weighed to the dry weight Wd of the tissue. Then the formula for calculating the relative water content of the blade is (Eq. (8)):

$$RWC = \frac{Wf - Wd}{Wt - Wd} \times 100\% \tag{8}$$

The osmotic potential was measured by an osmometer (Model 5520, Wescor, Logan, UT, USA). Each material was set to 3 replicates per process. The measured data was converted into international unified units (MPa) using the following formula (Eq. (9)):

$$\psi_s = \frac{cRT}{1 \times 10^6} \tag{9}$$

where, c: the osmometer indication; R: a constant, usually equal 8.314; T: Kelvin temperature, usually equal 298 K.

Proline content (Pro) was determined by the ninhydrin method [29], the regression equation was obtained from standard curve: according to the relationship between the absorbance value (y) and the valine concentration (x) of the standard solution at 520 nm. The sample absorbance value was calculated from the regression equation according to the regression equation (c ug/2 ml), and the percentage of proline content in the sample was calculated by Eq. (10):

$$Pro(ug \times g) = \frac{C \times \frac{5}{2}}{W} \tag{10}$$

where C: value found by standard curve, W: the fresh sample weight (g).

Soluble protein (sPro) content was determined by the Coomassie Brilliant Blue G-250 method. A standard curve was prepared by establishing a regression equation based on the standard solution absorbance value (y) at 595 nm and the soluble protein concentration (x). Soluble protein (*c*) (mg g^{-1}) (Eq. (11)):

$$c = \frac{C \times Vt}{Vs \times W \times 1000} \tag{11}$$

where C: the protein content (μg) got from the standard curve; Vt: the total volume of the extract; Vs: the volume of the extract used in the measurement (ml); W is the fresh weight of the sample (g).

2.4. Statistical analysis

The means of each treatment were compared with the least significant difference (LSD) test at the 0.05 level (p < 0.05). Regression analysis was used to analyze the relationships among the relative SWC with Pn and Gs. Logistic Curve fitted by Curve Expert 1.4

(Daniel Hyams). Data were collected by the Excel 2016, and analysis of variance (ANOVA) test was applied by using SAS (Version 8.0, SAS Inst., Cary, NC, USA) to evaluate the difference in each parameter.

3. Results

3.1. Effects of periodic drought-rehydration on photosynthetic rate and stomatal conductance of photosynthetic parameters

The photosynthetic rate (Pn) is a key parameter reflecting the vegetative growth of plants and their photosynthetic efficiency. The logistic curve illustrating Pn and soil water content (SWC) during the two drought processes is presented in Fig. 2(a). Overall, Pn values decreased gradually as soil water content diminished. Fitting the data revealed that Pn and relative soil water content followed a logistic equation ($R^2 > 0.98$), indicating that Pn exhibited a slow-rapid-slow pattern throughout the drought process. An over-compensation effect was observed during the two rounds of drought and rehydration. Under the same soil moisture content (54 %), Pn decreased by 7.88 % during the first round, while the second round showed a 10.12 % increase compared to the control group (CK), suggesting that drought-rehydration practices can enhance the photosynthetic rate of maize seedlings.

To quantify the compensation effects of the two drought processes, we analyzed the fitted logistic curve, specifically the area between the two inflection points (upper inflection point IP1 (IP'1) and lower inflection point IP2 (IP'2)), referred to as the rapid decline period. The magnitudes of changes in soil relative water content (Δx) and Pn (Δy) during this period are summarized in Table 1. The Δx in the rapid decline of soil water content during the first round was 11.24, while in the second round it was 10.01. This indicates that maize seedlings exhibited a more rapid response and greater drought tolerance following the second drought cycle. The corresponding changes in Pn for the two rounds were 5.81 and 7.58, respectively, demonstrating a strong overcompensation effect in maize seedlings after two rounds of drought. The ratio Δx/Δy was used to represent the response speed of Pn to drought across the two drought stages; higher values indicate a faster response. After two drought cycles, the calculated response speeds (k1 and k2) were 0.52 and 0.76, respectively, suggesting that cyclical drought exposure improved the seedlings' response speed to drought stress.

To further illustrate the super-compensation effect of drought-rehydration practices on maize seedlings, we analyzed stomatal conductance (Gs), as shown in Fig. 2(b). Gs also conformed to a logistic curve, with the second round showing an 11.13 % increase compared to the first round and a 5.61 % increase compared to CK. During the rapid decline period of the curve, Δx/Δy for the first round of drought was 2.95, while it increased to 3.21 in the second round. This further corroborates that cyclical drought-rehydration can produce an overcompensation effect on the photosynthetic performance of maize seedlings, with subsequent drought cycles enhancing their response speed to drought conditions.

3.2. Effect of periodic drought-rehydration on the change of osmosis regulation of seedling maize

The relative leaf water content (RWC) of maize seedlings subjected to two rounds of drought-rehydration treatment was measured, as shown in Fig. 3(a). On day 2 (D2), there was no significant difference between the treatment group (T) and CK. However, on day 5 (D5) of the first round, the RWC of the T group significantly decreased by 26.92 % compared to CK ($p < 0.05$). During the second round of drought-rehydration, RWC was significantly reduced by 40.22 % ($p < 0.01$) compared to CK on D5, but both groups returned to CK levels after 3 days of rehydration.

We further investigated osmotic regulation parameters and found that drought-rehydration practices significantly increased the osmotic potential of maize seedlings, as depicted in Fig. 3(b). There were no significant differences in osmotic potential between the T group and CK at D2 for either round. In contrast, the osmotic potential of the T group increased significantly by 45.69 % ($p < 0.01$) during the first round and by 40.16 % ($p < 0.01$) during the second round compared to CK at D5. After 3 days of rehydration, the
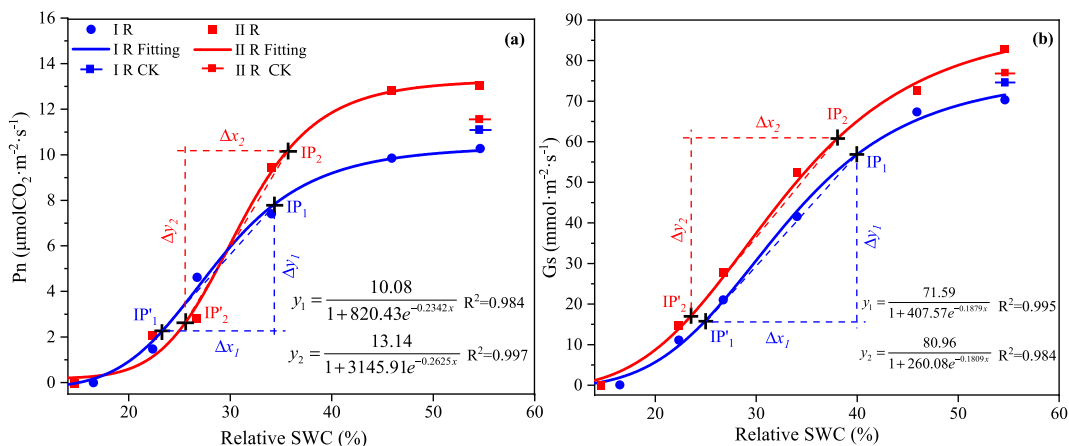
Fig. 2. Logistic curve fitting of soil relative water content and Pn (a) and Gs (b) value under two rounds of drought-rewatering treatment. IP and IP' (upper and lower Inflection Point respectively), I R (first round), II R (second round).

Table 1
The results of analyzed the fitting curve between IP and IP'

Parameter	Max_y		Δx		Δy		$\Delta x/\Delta y$	
	y_1	y_2	x_1	x_2	y_1	y_2	k_1	k_2
Pn	10.08	13.14	11.24	10.01	5.81	7.58	0.52	0.76
Gs	71.59	80.56	14.02	14.56	41.34	46.75	2.95	3.21
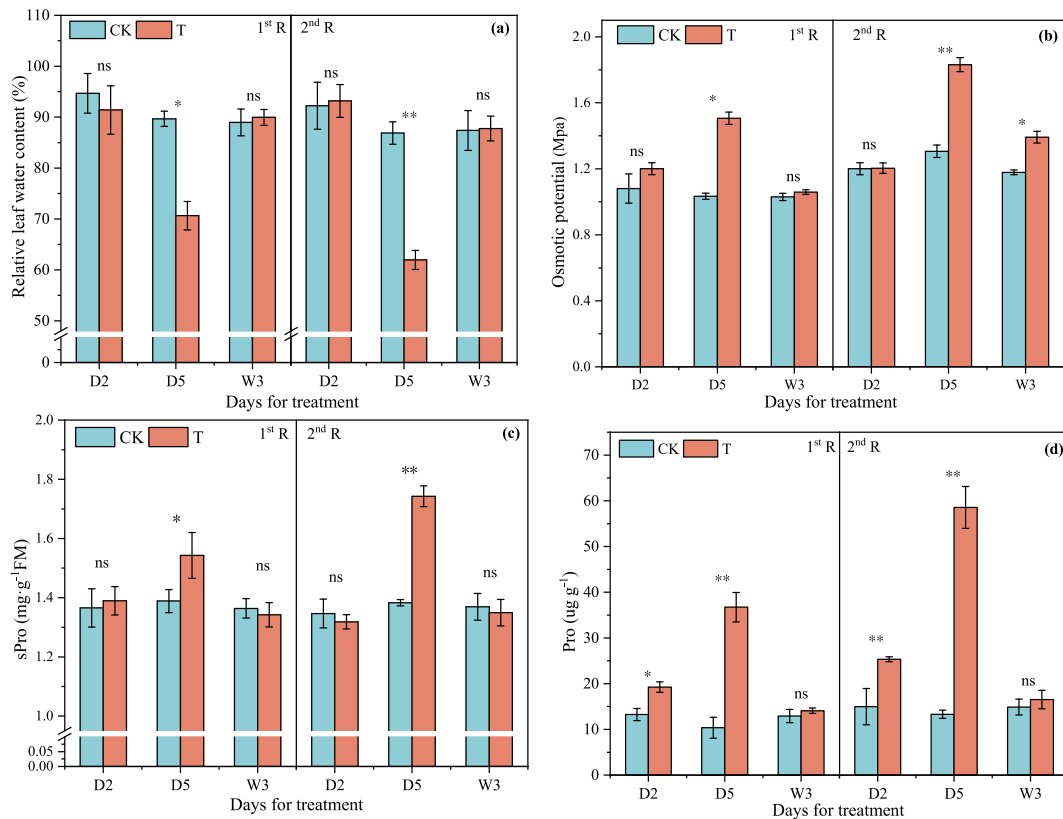
Fig. 3. Effect of periodic drought-rehydration on the change of osmosis regulation of maize. (a): relative leaf water content (RWC); (b): osmotic potential; (c): Soluble proteins (sPro); (d): proline (Pro) * is significantly difference at 0.05 level ($p < 0.05$), ** is significantly difference at 0.01 level ($p < 0.01$), ns is no significantly difference ($p < 0.05$).

Table 2
The contents of malondialdehyde and the active of antioxidant enzymes of seedling maize.

Round	Days	Treatment	MDA (μ mol g^{-1})	SOD (U g^{-1})	POD (U g^{-1} min^{-1})
First	D2	CK	6.03 ± 0.51 b	125.06 ± 14.25 a	2.78 ± 0.13 b
		T	7.92 ± 0.44 a	138.90 ± 19.71 a	3.22 ± 0.03 a
	D5	CK	6.56 ± 0.50 b	146.28 ± 29.40 b	2.81 ± 0.12 b
		T	10.87 ± 0.16 a	227.87 ± 31.45 a	3.52 ± 0.12 a
	R3	CK	7.05 ± 0.75 a	175.74 ± 16.62 a	3.03 ± 0.15 a
		T	7.58 ± 0.55 a	152.03 ± 21.91 a	3.13 ± 0.22 a
Sencond	D2	CK	7.06 ± 0.63 a	168.06 ± 35.29 b	2.94 ± 0.18 b
		T	7.75 ± 0.58 a	221.11 ± 27.90 a	3.43 ± 0.17 a
	D5	CK	7.36 ± 0.47 a	183.52 ± 23.86 b	2.89 ± 0.27 b
		T	7.88 ± 1.01 a	287.99 ± 17.90 a	4.19 ± 0.14 a
	R3	CK	7.14 ± 0.96 a	155.05 ± 27.41 a	2.96 ± 0.13 b
		T	7.07 ± 1.02 a	160.42 ± 25.55 a	3.26 ± 0.23 b

Data in the table are mean ± SD, and different lowercase letters means significant difference at 0.05 level ($p < 0.05$).

osmotic potential in both rounds returned to CK levels. Soluble proteins (sPro) and proline (Pro), critical osmotic-regulating substances, responded rapidly to drought stress to mitigate its effects (Fig. 3(c) and (d)). The content of sPro significantly increased by 11.16 % (p < 0.05) during the first round and by 26.12 % (p < 0.05) during the second round compared to CK at D5. Concurrently, Pro content increased significantly by 263.82 % (p < 0.01) and 343.49 % (p < 0.01) during the two rounds, respectively. After 3 days of rehydration, the levels of both sPro and Pro returned to CK levels. These results indicate that the drought-rehydration practice significantly enhances the response speed of sPro and Pro, thus improving the drought tolerance of maize seedlings.

3.3. Effects of rehydration on malondialdehyde and antioxidant enzymes in maize seedling

The changes in malondialdehyde (MDA) content and the activity of antioxidant enzymes (superoxide dismutase (SOD) and peroxidase (POD)) following two rounds of drought-rewatering are summarized in Table 2. During the first round of treatment, MDA content increased significantly by 65.70 % (p < 0.01) compared to the control group (CK) on day 5 (D5), indicating substantial oxidative stress in maize. Concurrently, the activities of SOD and POD increased significantly by 55.78 % (p < 0.05) and 25.26 % (p < 0.05), respectively, compared to CK. After three days of rehydration, all measured parameters returned to CK levels. In the second round of treatment, there was no significant difference in MDA content compared to CK throughout the experiment. However, SOD and POD activities increased substantially by 31.56 % and 16.67 %, respectively, on day 2 (D2), and were significantly higher by 56.93 % and 44.98 % than CK on D5. These results indicate that the drought-rewatering practice resulted in a pronounced compensatory effect, enhancing the antioxidant enzyme activity to protect maize plants from drought-induced damage.

3.4. Growth characteristics of maize seedling after two rounds of drought-rewatering practice

The growth characteristics of maize seedlings following two rounds of drought treatment are presented in Table 3. There were significant reductions in plant height, leaf area, and biomass compared to CK after the two rounds. Conversely, root length increased significantly by 21.05 % compared to CK, which is advantageous for enhancing drought resistance in maize seedlings. The survival rates were 91 % for the CK group and 84 % for the treatment group, indicating a positive outcome for the drought-rewatering practice.

3.5. Plant moisture threshold after two rounds of drought-rewatering practice

The theoretical relative soil water content (SWC) at which Pn equals zero was calculated through linear analysis, providing insights into the moisture thresholds that maize can tolerate during drought and rewatering treatments (Fig. 4). The lower limits of the moisture threshold were 16.5 % in the first round and 14.56 % in the second round, while the upper limits were 64.91 % and 66.29 %, respectively. The moisture threshold range increased by 7.19 % in the second round compared to the first round. This super-compensation effect observed after two rounds of drought practice suggests that maize seedlings can enhance their adaptability under lower water conditions following repeated drought exposure.

4. Discussion

Drought stress influences a series of physiological compensation effects at the physiological, biochemical, and molecular levels in crops [30]. The degree of these compensatory effects can vary depending on a combination of factors. Acevedo et al. [31] found that plants exhibit rapid growth following short-term water replenishment after experiencing water stress, indicating a compensatory or partial recovery mechanism [32]. This response may serve as an adaptive strategy for plants to cope with short-term, periodic, or unpredictable droughts [14], the effectiveness of this adaptive response correlates with the plants' overall resilience. In this study, we used maize (*Zea mays* L.) to investigate the drought resistance mechanisms in seedlings through repeated drought-rehydration treatments.

Photosynthesis is fundamental to crop development and yield formation and is particularly responsive to drought stress [33,34]. For instance, I. Allahverdiyev [35] reported that drought significantly reduces photosynthetic rates and stomatal conductance. DaMatta et al. [6] noted that severe drought could decrease crop carbon assimilation by 68–80 %, consistent with our findings that Pn in maize seedlings was significantly higher after the first round of drought-rewatering compared to the control group. Additionally, Pn and stomatal conductance (Gs) increased substantially in the second round compared to the first, demonstrating a super-compensation effect. This phenomenon can be attributed to the production of abscisic acid during the first round of drought stress, which reduces stomatal opening. The plants develop adaptive resistance mechanisms and gene expression, effectively allowing them to "remember" previous drought conditions. This memory enables them to promptly close their stomata during subsequent drought periods to

Table 3
Growth characteristics of maize seedlings after two rounds of drought-rewatering.

Treatment	Plant height cm	Leave Area cm^2 plant^{-1}	Total root length mm·plant^{-1}	Biomass g·plant^{-1}	survival rate %
CK	61.3 ± 3.1 a	104.21 ± 3.4 a	858.28 ± 28.3 b	3.78 ± 0.34a	91 ± 2.1 a
T	53.4 ± 2.5 b	84.78 ± 2.8 b	1038.91 ± 32.6 a	2.51 ± 0.27 b	84 ± 2.5 b

Data in the table are mean ± SD, and different lowercase letters mean significant difference at 0.05 level (p < 0.05).
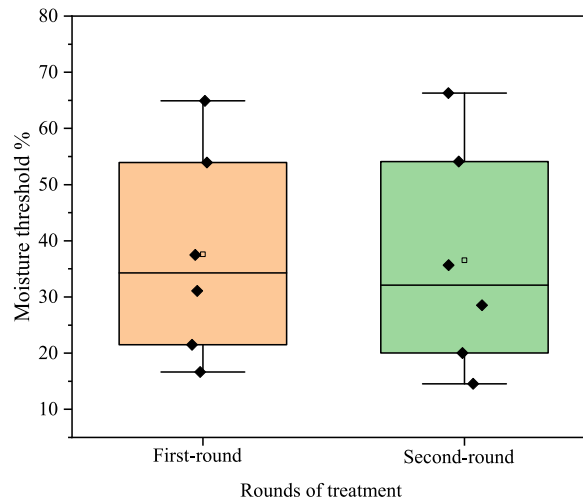
Fig. 4. Soil moisture threshold in two rounds of treatment.

minimize water loss. After rehydration, both Pn and Gs returned to control levels, indicating that maize can recall past drought experiences, and respond swiftly when faced with drought stress again [36,37].

In addition to adapting through changes in photosynthetic morphology, plants also employ osmotic adjustment mechanisms to mitigate water loss during drought stress [38]. Soluble proteins accumulate following drought-rehydration, which can lower osmotic potential, and help maintain turgor pressure, thereby enhancing drought resistance [17,22,34]. Continuous cycles of drought and rehydration can improve the responsiveness of the internal adjustment systems of plants under drought stress, thus enhancing crop drought resistance [39]. In our study, soluble protein content increased by 14.96 % on the fifth day of the second round of drought treatment, while osmotic potential decreased by 5.53 % compared to the first round. Although leaf moisture content was significantly lower than that of the control group after 5 days of drought treatment in the second round, it returned to control levels following rehydration, suggesting the presence of drought memory. This indicates that drought practice can enhance the sensitivity of osmotic adjustment mechanisms in crops [40].

SOD, POD and other antioxidant enzymes play crucial roles in protecting plants from oxidative damage [41,42]. Studies have shown that the activity of antioxidant enzymes significantly increases in response to elevated MDA levels caused by drought stress [43]. In our study, both SOD and POD activities increased significantly throughout the drought conditions, whereas leaf osmotic potential only increased on day 5. Following three days of rehydration, the levels of SOD, POD, and MDA returned to control levels, indicating that maize seedlings recovered swiftly after drought exposure, demonstrating a super-compensatory effect. Chen and Jiang [44] reported that drought treatments enhance the response speed of antioxidant activity, a finding supported by our results, which showed that SOD and POD activities were significantly greater in the second round than in the first round. This suggests that repeated drought-rehydration practices positively influence the activity of antioxidant enzymes. Relative water content (RWC) in plants decreases rapidly under drought stress, leading to increased membrane permeability and MDA accumulation [41,45]. MDA content serves as an indicator of plant damage, with elevated levels stimulating antioxidant enzymes to restore balance; this mechanism is relatively less effective under normal conditions. Our research indicates that the regulatory effects of antioxidant enzymes can be enhanced through appropriate drought practices, allowing plants to "remember" previous drought experiences and respond more rapidly under similar conditions [46]. In some cases, this rapid response may even result in a super-compensatory effect, promoting plant growth.

The growth characteristics of plants-such as leaf area, root length, plant height, and biomass-serve as indicators of drought tolerance [4]. When experiencing drought stress, plants adapt by either absorbing water from deeper soil layers or reducing their own water consumption. The root system, which is directly related to soil conditions, significantly impacts the growth and development of aerial parts and grain formation [47]. Conversely, leaf morphology—acting as the primary site for photosynthesis and transpiration—adapts to drought conditions by becoming smaller and thicker; similar changes are observed in plant height, which is considered a protective response to water stress [48]. These adaptive features can be leveraged through drought training to stimulate super-compensatory effects, thereby enhancing drought resistance. In our study, after two rounds of drought practice, plant height and leaf area decreased, while root length increased significantly compared to the control group. Guo et al. [49] noted that drought training during the seedling stage is particularly beneficial for wheat, with trained plants exhibiting enhanced stress resistance. Our findings corroborate this, revealing that while plant height, leaf area, and biomass decreased to some extent compared to the control group after two rounds of drought training, the significant increase in root length represents an adaptive benefit for the plants in resisting drought stress.

5. Conclusion

This study investigated the drought adaptability of seedling maize through two rounds of drought-rewatering treatment. We found that photosynthesis exhibited a logistic relationship ($R2 > 0.98$) with relative soil water content (SWC), demonstrating a significant super-compensatory effect: photosynthetic rate (Pn) and stomatal conductance (Gs) increased by 10.12 % and 5.61 %, respectively, compared to the control group (CK). Although osmotic parameters on day 5 (D5) of both drought rounds were significantly lower than those of CK, all measured factors returned to control levels after the two rounds of treatment. The activities of antioxidant enzymes, including SOD, POD, and CAT, positively contributed to the reduction of MDA content, further indicating a super-compensatory effect. Plant morphology, which reflects drought stress, showed decreased values for plant height, leaf area, and biomass after the drought practice, while root length increased significantly. Overall, the drought-rewatering practice resulted in a positive super-compensatory effect, enhancing the drought resistance of seedling maize and improving its adaptability to arid environments. These findings are important for optimizing population dynamics, structural integrity, and yield formation in maize cultivation.

CRediT authorship contribution statement

Chenkai Gao: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pengnian Wu:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Yanli Wang:** Supervision, Software, Resources, Methodology, Investigation. **Pengfei Wen:** Validation, Supervision, Methodology, Conceptualization. **Xiaokang Guan:** Validation, Supervision, Methodology. **Tongchao Wang:** Validation, Supervision, Software, Resources, Conceptualization.

Data availability

Data will be made available on request.

Declaration of competing 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.

Acknowledgement

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