A study on soybean responses to drought stress and rehydration

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1. Introduction

Soybeans are an important crop that needs a sufficient water supply during its growth process to achieve high yields (Buezo et al., 2019). Drought stress, however, does not only cause damage to the plants. After a certain level of drought stress, and for a short period after rehydration, soybean plants can exhibit positive physico-biochemical growth compensation or overcompensation in terms of their metabolism and their growth and development to compensate for the damage and losses caused during drought stress (Liu et al., 2012; Hao et al., 2010; Xue et al., 2013). Drought stress can significantly reduce the chlorophyll a, b, and total chlorophyll contents (Wu and Zhang, 2019). A compensation effect is commonly present in plants and is often produced after injury or stress (Dai, 2007). Compensation is an important self-regulatory mechanism used by plants to defend against environmental stresses or injuries. It is also a major physiological reference for effective water control in plants and an indicator of highly water-efficient agriculture (Luo et al., 2013; Li et al., 2014). An

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Abstract
To investigate soybean responses to drought stress and growth through metabolism compensation after rehydration, and for the establishment of an optimal water-saving irrigation model, we used the soybean variety Suinong 14 as experimental material and adopted a weighing method for water control in potted plants. We exposed soybean plants to stress treatments at different growth stages using different stress levels and durations. We then studied the effects of drought stress and rehydration on soybean growth and development, osmoregulation, and endogenous hormonal regulations, as well as antioxidant systems. The results showed that drought stress inhibited increases in the soybean plant height and leaf area. This inhibition became more significant as the level, duration, and frequency of the drought stress increased. After rehydration, the soybean plant heights and leaf areas exhibited rapid increases and partial compensation for their decreased sizes. As the level, duration, and frequency of drought stress increased, the compensation effect decreased, but it did not return to the control level. Drought stress reduced the chlorophyll content and relative water content in the soybean leaves and increased the osmolyte contents, antioxidant potential, and peroxidation of the membrane lipids. In addition, the changes mentioned above became more dramatic as the drought stress level, duration, and frequency increased. Upon rehydration, various levels of growth compensation were observed in each physio-biochemical parameter. As the drought stress level, duration, and frequency increased, the compensation effect also increased. Overall, the compensation effect for drought stress that occurred at the early growth stages was higher than that at the later growth stages. Drought stress led to decreases in the ZR/IAA and ZR/ABA ratios in soybean leaves and an increase in the ABA/(IAA + GA + ZR) ratio; thus, the plant growth was inhibited. These hormone ratios exhibited more dramatic changes when the drought stress level became more severe and the stress duration was prolonged. After rehydration, these hormone ratios produced equal compensation effects. Therefore, the compensatory effect of rewatering after drought stress is conditional. Severe stress, especially long-term severe stress, will reduce the compensatory effect. At the same time, drought resistance treatment at seedling stage can improve the adaptability and compensation effect of re-drought at grain filling stage.

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obvious example of compensation lies in the plant external morphology after a stress is relieved, such as short and rapid growth in the plant height, leaf area, and growth rate, i.e., the growth compensation effect (Bu et al., 2009). Plant growth and development are regulated by a coordination of various endogenous hormones and physio-chemical metabolism. Endogenous hormones are the most important regulators throughout the plant's life cycle, and they play roles in transducing stress signals when plants are under stress. Through antagonistic regulations among endogenous hormones, these hormones regulate the plant physio-chemical metabolism, growth and development to promote plant adaptation to a stressful environment (Ha et al., 2012; Wang et al., 2004). Osmoregulation is an important characteristic of plant drought resistance in which the content of malondialdehyde (MDA), a product of membrane lipid peroxidation that is produced when plants are under stress, can reflect the degree of cell membrane damage (Li and Nong, 2018; Wang et al., 2019). The antioxidant system can actively adapt, control, and remove reactive oxygen species (ROS). For example, when soybeans are under water stress, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) usually increase, and the MDA content also significantly increases at the same time (Wu et al., 2019). Studies have shown that drought stress and rehydration could lead to a growth compensation effect, including an increase in the plant height, the accelerated growth of the leaves, and an increased rate of dry matter accumulation (Wu et al., 2019; Hao et al., 2010; Li et al., 2013; Liang et al., 2011; Gao et al., 2007). During this process, changes in the ratios of different endogenous hormone contents would result in different physiological reactions that regulate the growth and development of plant organs. Through high levels of SOD, APX, and CAT enzyme activity, ROS can be effectively removed to mitigate membrane damage and membrane lipid peroxidation. In addition, antioxidants that accumulate in the cells, such as proline, soluble sugars, and MDA, rapidly decrease (Wang et al., 2012; Luo et al., 2014) and ultimately confer a post-drought compensation effect. In this study, we performed water control by weighing method to decrease the amount of irrigation applied to the Suinong 14 soybean variety gradually. The plants were subjected to drought stress at different levels and durations at the seedling stage, seed-fill stage, and seedling stage + seed-fill stage, followed by rehydration. Using this approach, we studied the effect of drought stress and rehydration on the leaf growth compensation effect, endogenous hormone regulation, osmoregulation, and the antioxidant system (Li et al., 2019). We investigated the mechanism of the growth compensation effect in response to drought stress and rehydration in depth. Our goal was to provide a theoretical basis for the mechanism of compensation in response to drought and to help determine the direction of high-yield, drought-resistant soybean cultivation technologies.

2. Materials and methods

2.1. Experimental materials

The experiments were conducted in the rain shelter located at the Botany Experiment and Practice Base of Northeast Agricultural University, China.

Table 1

| Total Nitrogen g kg⁻¹ | Total Potassium g kg⁻¹ | Total Phosphorus g kg⁻¹ | NO₃⁻N mg kg⁻¹ | NH₄⁺-N mg kg⁻¹ | Available potassium mg kg⁻¹ | Available phosphorus mg kg⁻¹ | Organic Matter g kg⁻¹ |
|-----------------------|------------------------|-------------------------|---------------|----------------|-----------------------------|-----------------------------|----------------------|
| 1.40                  | 55.52                  | 0.61                    | 46.29         | 32.48          | 148.84                      | 11.19                       | 23.52                |

The variety used in the experiment was Suinong 14. The soil used in the experiment was topsoil from the Botany Experiment and Practice Base of Northeast Agricultural University; the soil type was black soil, and the soil capacity was 34.01%. The soil was air-dried after sifting. The basic soil fertility data are shown in Table 1.

The potting materials were plastic pots (with holes at the bottom) measuring 33 cm high with a 26 cm inner diameter. A PVC tube (2 cm diameter, 40 cm length) attached to a plastic funnel was placed in the pot, and small holes were poked in the tube. The tube was buried 5–7 cm underneath the seeds, with the funnel above the soil surface. Care was taken to ensure uniform irrigation to reduce surface evaporation and to prevent surface soil caking.

The rain shelter for the experiment consisted of a reinforced steel frame and glass on the top. It was cleaned regularly to ensure sufficient sunlight inside the shelter. Mesh nylon nets were used on the sides for good shelter air circulation.

2.2. Experimental design

The following three factors were part of the pot experiment: the plant growth stages under challenges from stress, different stress levels, and different stress durations, for a total of 18 treatments. Three plant growth stages were set up under stress as follows: a seedling stage (V3) and a seed-fill stage (R5) with one stress treatment during the entire growth period, and a seedling stage + seed-fill stage (V3R5) with stress treatments occurring twice during the entire growth period. Different stress levels were set up in the following 3 treatments: sufficient water supply (CK), the relative water content of the soil was 75–80% soil capacity), moderate stress (S, the relative water content of the soil was 60–65% soil capacity), and severe stress (SS, the relative water content of the soil was 45–50% soil capacity). The stress durations were 5 d and 10 d, for a total of 2 levels, as shown in Table 2. CK-V3-5 and CK-V3-10 were the controls for 5 d and 10 d of drought stress at the V3 stage, respectively. CK-R5-5 and CK-R5-10 were the controls for 5 d and 10 d of drought stress in the R5 and V3 + R5 stages, respectively.

Potted plants were used for the experiment. Each pot contained 13 kg of soil that was fertilized with 2.0 g/pot diammonium phosphate (equivalent to 150 kg/ha⁻¹), 1.5 g/pot triple superphosphate (P₂O₅: 46%) (equivalent to 150 kg/ha⁻¹), and 1.5 g/pot potassium sulfate (K₂O: 30%) (equivalent to 150 kg/ha⁻¹). Fertilizers were applied 5–7 cm below the seeds. No more fertilizer was added during the entire growth period. On May 11th, carefully selected seeds were sown into shallow holes that were poked into the soil. In each pot, 6 holes were made at equal distances and 3 seeds were sown in each hole. A 2–3 cm layer of dry soil was used to cover the seeds after sowing. All the seeds were germinated, and all grew into uniform seedlings. Following thinning, 3 young soybean seedlings with strong growth vigour and similar growth characteristics were saved (1 seedling in each hole, for a total of 3 holes in each pot). Water control was performed by gradually reducing the irrigation amount until the relative water content of the soil reached the expected value. Using a weighing method, the weight of each pot was recorded once every 2 days. The amount of irrigation needed was then calculated. When the relative water content of the soil was lower than the lower limit of the water control treatment,
water was added precisely through the funnel to the upper limit; in this way, the drought stress gradient was maintained (Li et al., 2019). When the stress duration was completed, rehydration was performed on the same day to the control level. After that, a sufficient water supply was maintained until harvest. Except for the water control, the other cultivation and management measures were the same as the regular field management measures.

### 2.3. Sampling method

Samples were taken on the day of rehydration, as well as Day 5 and Day 10 after rehydration. The sampling time was 8:00–9:00 AM. Before sampling, the potted plants were removed from the rain shelter and placed in the sunlight for 1–2 h. Three plants with uniform growth characteristics were selected from each treatment, and the top second and third leaves were harvested, mixed, and divided into 5 samples. Each treatment had 3 replicates (Li et al., 2018). The samples were divided into 2 portions; one portion was kept on ice for immediate measurement and the other portion was flash-frozen in liquid nitrogen and stored in a −80°C freezer.

### 2.4. Measuring items and methods

#### 2.4.1. Determination of morphological indicators

2.4.1.1. Plant height. Straight ruler method was used to determine the plant in vivo. Each treatment was fixed in three basins. Records were made on the day of rehydration and on the 5th and 10th days after rehydration, and once during harvest and delivery.

2.4.1.2. Leaf area. The length–width coefficient method was used to determine the plant in vivo. Each treatment was fixed in three basins and recorded once on the day of rehydration and on the 5th and 10th days after rehydration, and once during harvest and delivery (Li, 1978).

#### 2.4.2. Determination of physiological and biochemical indexes

2.4.2.1. Relative water content of leaves (RWC). The middle part of fresh leaves was weighed about 0.1 g, weighed fresh (Wf), soaked in distilled water for 12 h, weighed saturated (Wt), then put into oven at 105°C for 10–15 min, and then dried to constant weight (Wd) at 80°C (Li, 2003).

\[
\text{Calculating formula: } \text{RWC} = \frac{W_f - W_d}{W_t - W_d} \times 100\%
\]

2.4.2.2. Relative permeability of plasma membrane. The relative conductivity (RC) of the blade was measured by conductometer. 10 fresh blades were sampled with a perforator, and then rinsed with deionized water three times, put in a conical bottle with deionized water 20 mL, put in a vacuum drying vessel, exhaust for 1 h, and put at room temperature for 24 h. The conductivity R1 was measured by DDB-303A portable conductometer. The conical bottle was heated in a boiling water bath for 15 min, then cooled to room temperature, and its conductivity R2 was measured (Hao, 2004).

\[
\text{Calculating formula: } RC = \frac{R1}{R2}
\]

2.4.2.3. Chlorophyll content. SPAD-502 chlorophyll meter was used to measure the relative chlorophyll content (SPAD value) of the last three leaves in vivo, fixed in three pots for at least five times, and recorded on the day of rehydration and on the 5th and 10th days after rehydration.

2.4.2.4. Superoxide dismutase activity (SOD). Using colorimetric method, 0.2 g fresh leaves of plants were cut up, and in the pre-cooled mortar, 2 mL pre-cooled phosphate buffer solution (PBS, PH 7.8) and a small amount of quartz sand were added to grind into homogenate in ice bath. Then, 2 mL and 1 mL pre-cooled phosphate buffer solution were used to rinse the mortar once, respectively, and the rinse solution was combined into the centrifugal tube, and centrifuged for 15 min at 4°C for 10 000 r/min. The supernatant was SOD. Extract. Phosphoric acid buffer solution (PBS, PH 7.8) 1.5 mL, methionine (Met) 0.3 mL, nitrogen blue tetrazole (NBT) 0.3 mL, EDTA-Na 20.3 mL, riboflavin 0.3 mL, SOD extract 0.1 mL and distilled water 0.5 mL were added in turn to the transparent glass tube. The tube was placed under 4000 LX fluorescent lamp for photochemistry reaction for 20 min, and the temperature was controlled between 25 and 35°C. The cloth mask was used to cover the termination reaction of the test tube. The control tube was set to zero in the dark, and distilled water was used instead of enzymatic solution as control. The absorbance value of the reaction solution was measured at 560 nm. The amount of enzyme required to inhibit 50% photochemistry of NBT was a unit of enzyme activity (U), and U g⁻¹FW min⁻¹ was used to express the activity of enzyme (Gao, 2006).

### Table 2

| NO. | Treatment Symbol                | Stree levels     | Stree days | Symbol |
|-----|--------------------------------|-----------------|------------|--------|
| 1   | Seedling stage (V3)             | Adequate water supply | –          | CK-V3-5 |
| 2   | Moderate stress                 | Moderate stress  | 5 d        | V3SS5  |
| 3   | Severe stress                   | Moderate stress  | 5 d        | V3SSS5 |
| 4   | Adequate water supply           | Moderate stress  | 10 d       | V3S10  |
| 5   | Severe stress                   | Moderate stress  | 10 d       | V3SS10 |
| 6   | Adequate water supply           | Adequate water supply | –          | CK-R5-5 |
| 7   | Moderate stress                 | Moderate stress  | 5 d        | R5S5   |
| 8   | Severe stress                   | Moderate stress  | 5 d        | R5SS5  |
| 9   | Adequate water supply           | Moderate stress  | 10 d       | R5S10  |
| 10  | Severe stress                   | Moderate stress  | 10 d       | R5SS10 |
| 11  | Seed filling stage (R5)          | Adequate water supply | –          | CK-R5-5 |
| 12  | Moderate stress                 | Moderate stress  | 5 d        | R5S5   |
| 13  | Severe stress                   | Moderate stress  | 5 d        | R5SS5  |
| 14  | Adequate water supply           | Moderate stress  | 10 d       | R5S10  |
| 15  | Severe stress                   | Moderate stress  | 10 d       | R5SS10 |
| 16  | Adequate water supply           | Adequate water supply | –          | CK-R5-5 |
| 17  | Moderate stress                 | Moderate stress  | 5 d        | R3S5   |
| 18  | Severe stress                   | Moderate stress  | 5 d        | R3SS5  |

Note: “−” Expressed no.
Calculating formula: SOD activity (U·g⁻¹ FW·min⁻¹)
\[
\frac{(A_0 - A_s) \times V_t}{A_0 \times 0.5 \times W \times V_s \times t}
\]
Formula: \(A_0\): absorbance of control tube under light; \(A_s\): absorbance of sample determination tube; \(V_t\): total volume of sample extract, mL; \(V_s\): crude enzyme volume, mL; \(t\): color reaction time, min; \(W\): fresh weight of sample, g.

2.4.2.5. Peroxidase activity (POD). Using colorimetric method, 0.2 g fresh leaves of plants were cut and added into the pre-cooled mortar, 2 mL pre-cooled phosphoric acid buffer solution (PBS, PH 6.0) and a small amount of quartz sand were ground into homogenate in ice bath, then rinsed with 2 mL and 1 mL pre-cooled phosphoric acid buffer solution respectively once, combined with the rinse solution into the centrifugal tube, and centrifuged for 15 min at 4 °C. The supernatant was extracted as POD. Take liquid. In the colorimetric cup, 3 mL of reaction mixture (reaction mixture: 100 mmol/L phosphate buffer (PH 6.0) and 50 mL of guaiacol 28 mL and 30% hydrogen peroxide 19 mL were added to mix evenly). When the activity of POD was too high and could be diluted, the phosphoric acid buffer solution replaced the enzyme solution as a control. The stopwatch recording time was immediately turned on. The absorbance value of the reaction solution was measured at 470 nm and read every 30 s. The change of A470 per minute was 0.01 as a unit of peroxidase activity (U) (Zhang, 2004).

Calculating formula: POD (U·g⁻¹ FW·min⁻¹)
\[
\frac{\Delta A_{470} \times V_t}{W \times V_s \times 0.01 \times t}
\]
Formula: \(\Delta A_{470}\): change of absorbance in reaction time; \(V_t\): total volume of extracting enzyme solution, mL; \(V_s\): volume of enzyme liquid, mL; \(W\): fresh weight of sample, g; \(t\): reaction time, min.

2.4.2.6. Catalase activity (CAT). Using colorimetric method, 0.2 g fresh leaves of plants were cut, and in the pre-cooled mortar, 2 mL pre-cooled phosphoric acid buffer solution (PBS, PH 7.0) and a small amount of quartz sand were added to grind into homogenate in ice bath. Then, 2 mL and 1 mL pre-cooled phosphoric acid buffer solution were used to rinse the mortar once, respectively. The rinse solution was combined into the centrifugal tube, and centrifuged for 15 min at 4 °C. The supernatant was extracted by CAT. Take liquid. The tube was prepared by adding 0.2 mL of CAT extract, 1.5 mL of phosphate buffer and 1.0 mL of distilled water in turn. After preheating at 25 °C, adding 20.3 mL of hydrogen peroxide, the tube was quickly poured into the colorimetric cup and the time was started. The absorbance value of the reaction solution was measured at 240 nm. The absorbance value was read every 1 min for 4 min. The dead enzyme solution was boiled in boiling water as a control. The decrease of A240 by 0.1 in 1 min was expressed as a unit of enzyme activity (U) (Zhang, 2004).

Calculating formula: CAT (U·g⁻¹ FW·min⁻¹)
\[
\frac{-\Delta A_{240} \times V_t}{0.1 \times V_s \times t \times FW}
\]
Formula: \(\Delta A_{240}\): difference of absorbance between sample tube and boiled tube; \(V_s\): total volume of extracting enzyme solution, mL; \(V_s\): volume of enzyme liquid, mL; \(W\): fresh weight of sample, g; \(t\): reaction time, min.

2.4.2.7. Malondialdehyde content (MDA). Using thiobarbituric acid colorimetric method, 0.2 g leaves of plants were cut and ground into homogenate in a pre-cooled mortar with 10% trichloroacetic acid (TCA) 2 mL and a small amount of quartz sand in an ice bath. The mortar was rinsed once with 2 mL and 1 mL 10% TCA, respectively, and the rinse solution was combined into a centrifugal tube. The supernatant was taken as MDA extract at 4 °C for 4000 r/min after centrifugation for 10 min. MDA extract 2 mL was absorbed into test tube, 0.6% thiobarbituric acid (TBA) 2 mL was added, heated in water bath for 15 min, cooled rapidly and centrifuged again. The optical density of supernatant was measured at 532, 600 and 450 nm wavelengths. The content of malondialdehyde was calculated by zeroing the distilled water (Zhang et al., 2005).

Calculating formula: MDA (μmol/g) = \[\frac{C \times N}{W}\]
Formula: \(C = 6.45(D_{532}-D_{600})-0.56 D_{450}\), micron oL/L; \(N\): total volume of extract, mL; \(W\): sample weighing, g.

2.4.2.8. Soluble sugar content (WSS). Using thiobarbituric acid colorimetric method, 0.2 g leaves of plants were cut and ground into homogenate in a pre-cooled mortar with 10% trichloroacetic acid (TCA) 2 mL and a small amount of quartz sand in an ice bath. The mortar was rinsed once with 2 mL and 1 mL 10% TCA, respectively, and the rinse solution was combined into a centrifugal tube. The supernatant was taken as MDA extract at 4 °C for 4000 r/min after centrifugation for 10 min. MDA extract 2 mL was absorbed into the test tube, 0.6% thiobarbituric acid (TBA) 2 mL was added, heated in a water bath for 15 min, cooled rapidly and centrifuged again. The supernatant was taken to determine the optical density at 450 nm wavelength. The content of soluble sugar was calculated by zeroing the distilled water (Wang et al., 2003).

Calculating formula: WSS (mg/g) = \[\frac{C \times M}{W}\]
Formula: \(C = 11.7 D_{450}\) mmol/L; \(M\): molar mass, g/mol; \(W\): sample weighing, g.

2.4.2.9. Soluble protein content. Using Coomassie Brilliant Blue G-250 staining method, the leaves of 0.2 g were cut and ground in a pre-cooled mortar with distilled water of 2 mL and a small amount of quartz sand in an ice bath. The mortar was rinsed with distilled water of 2 mL and 1 mL respectively. The rinse solution was combined into a centrifugal tube and centrifuged for 10 min at 4 °C for 5000 r/min. The supernatant was the protein extract. Accurate extraction of protein was 0.1 mL, 0.9 mL distilled water and 5 mL Coomassie Brilliant Blue G-250 reagent were added. Fully blended, colorimetric analysis was performed at 595 nm wavelength after 2 min. Absorption value was recorded and protein content was determined by standard curve (Hao, 2007).

Calculating formula: soluble protein (mg/g) = \[\frac{C \times V_t}{V_s \times W \times 1000}\]
Formula: \(C\): from the standard curve, we can find that, ug; \(V_t\): the total volume of extracting enzyme solution, mL; \(V_s\): the volume of extracting liquid, mL; \(W\): fresh weight of sample, g; \(1000\): the total volume of extracting enzyme solution, mL; \(V_s\): the volume of extracting liquid, mL; \(W\): the fresh weight of sample, g; \(1000\): the conversion of ug into mg.

2.4.2.10. Proline content (Pro). 0.2 g leaf material was weighed by acid hydration ninhydrin coloration method. Cut it and put it into plugged test tube. Add 5 mL of 3% sulfosalicylic acid solution, then extract it in boiling water bath for 10 min. Directly absorb and extract 2 mL of clear night in plugged test tube, add 2 mL distilled water, 2 mL glacial acetic acid and 4 mL acetic ninhydrin, shake well, then heat it in boiling water bath for 60 min, cool it to room temperature, add 4 mL toluene, shake well to extract red substance. After extraction, it was separated into layers by stationary position. After fully stratified, toluene layer was absorbed and
the absorbance value was determined at 520 nm wavelength (Hao, 2007).

Calculating formula \[ \text{Pro}(\mu g/g) = \frac{C \times V}{W} \]

Formula: C: found by standard curve, \( \mu g \); V: total volume of extract, mL; a: liquid volume, mL; W: fresh weight of sample, g.

2.4.2.11. Endogenous hormone content. The contents of abscisic acid (ABA), auxin (IAA), gibberellin (GA) and Zeatin nucleoside (ZR) in soybean leaves were determined by enzyme-linked immunosorbent assay (ELISA). The test kit was provided by Qisong Biotechnology Co., Ltd (He, 1993).

1. Extraction of Hormones from Samples
   (1) Take 0.5 g leaf material, add 2 mL sample extract (80% methanol, containing 1 mmol/L BHT/di-tert-butyl-p-cresol), grind it into homogeneous slurry under ice bath, put into 10 mL test tube, then rinse it with 2 mL extract in succession, then turn it into test tube, shake it evenly and place it in 4 °C refrigerator.
   (2) Extract at 4 °C for 4 h, centrifuge at 1000 r/min for 15 min, and then take it out at night. 1 mL of extract was added to the precipitation, shake well, put at 4 °C, extract for 1 h, centrifuge, merge supernatant, and discard the residue.
   (3) Shangqing night C-18 solid phase extraction column. The specific steps are as follows: 80% methanol (1 mL) equilibrium column sampling sample collection sample removal and column washing with 100% methanol (5 mL) 100% ether (5 mL) 100% methanol (5 mL) column washing cycle.
   (4) After passing through the column, the sample was transferred into a 5 mL plastic centrifugal tube. The nitrogen was dried, the methanol in the extract was removed, and the volume of the sample diluent was fixed at 1 mL (Sample diluent: 500 mL phosphate buffer solution added 0.5 mL Tween, 0.5 g gelatin, slightly heated to dissolve).

2. Sample determination
   (1) Remove the required strips from the aluminium foil bag after 20 min of room temperature balance, and seal the remaining strips back to 4 °C with a self-sealing bag.
   (2) Standard pore and sample pore are set up, and standard pore is added 50 \( \mu L \) of different concentration of standard pore.
   (3) Sample holes are first added with 10 \( \mu L \) of the sample to be tested, followed by 40 \( \mu L \) of the sample diluent; blank holes are not added.
   (4) In addition to the blank pore, the standard pore and the sample pore were incubated with HRP-labeled antibody of 100 \( \mu L \) per pore, and the reaction pore was sealed with a sealing plate membrane. The reaction pore was incubated in a 37 °C water bath or thermostat for 60 min.
   (5) Discard liquid, pat dry on absorbent paper, fill each hole with detergent, stand for 1 min, shake off detergent, pat dry on absorbent paper, so wash the board five times (or wash the board with a washing machine).
   (6) Substrates A and B were added to each pore for 50 \( \mu L \) and incubated at 37 °C for 15 min.
   (7) The OD values of each pore were measured at 450 nm wavelength within 15 min after adding terminating solution 50 \( \mu L \) to each pore.

3. Result calculation

Calculating formula \[ A = \frac{N \times V \times B \times M}{W} \]

Among them, A: hormone content, ng/g; N: hormone concentration, pmol/L; V: constant volume, mL; B: dilution multiple; M: molar mass, g/mol; W: sample weight, g.

2.5. Data analysis

SPSS 17.0 and Excel 2003 were used for data collation and statistical analysis.

2.6. Comprehensive assessment of the antioxidant potential in leaves

Under drought stress and rehydration, plants exhibit their antioxidant potential through the synergistic effects of the protective enzyme system. Therefore, it is not possible to determine the precise antioxidant potential based on changes in only one antioxidant enzyme (Liang et al., 2008). The subordinate function method is used to assess multiple measured parameters comprehensively. In this study, the subordinate function method was adopted to assess the antioxidant potential comprehensively in soybean leaves that were subjected to drought stress at different growth stages followed by rehydration (Han et al., 2008; Yang et al., 2005; Xie et al., 2008).

The formula for calculating the value of membership function is as follows:

\[ F_j^* = \left( F_j - F_{j \text{min}} \right) / \left( F_{j \text{max}} - F_{j \text{min}} \right), \quad \bar{F}_i = \frac{1}{n} \sum_{j=1}^{n} F_j^* \]

In the formula, \( F_j \) is the measured value of an index of drought stress treatment, \( F_{j \text{max}} \) is the maximum value of the index, and \( F_{j \text{min}} \) is the minimum value of the index. \( F_j^* \) is the subordinate value of \( j \) index of drought stress treatment. \( \bar{F}_i \) is the membership function value (MV) of I drought stress treatment, and n is the index number.

If one parameter was negatively correlated with the antioxidant potential in the soybean leaves, the reverse subordinate function method was used for conversion.

The calculation formula is as follows:

\[ F_j^* = 1 - \left( F_j - F_{j \text{min}} \right) / \left( F_{j \text{max}} - F_{j \text{min}} \right), \quad \bar{F}_i = \frac{1}{n} \sum_{j=1}^{n} F_j^* \]

The membership value was calculated using three parameters, namely, the SOD, POD, and CAT.

3. Results and analysis

3.1. The effect of drought stress and rehydration on soybean morphology

Plant morphological characteristics directly reflect the growth and development of crops. Among them, the plant height is one of the most important indicators of plant growth and development, and it reflects the growth rate. The leaf area is also an important parameter related to the amount of light energy captured by crops, and therefore it directly affects photosynthesis, transpiration, and the final yield. Studying plant morphological characteristics can help researchers to determine the effects of drought stress and rehydration on soybeans as well as their changing patterns.

Tables 3 and 4 show that drought stress inhibited the growth of the plants in terms of height and leaf area. As the stress level worsened and the stress duration was prolonged, this inhibitory effect became more significant. Upon rehydration after 5 d of stress at the V3 stage, there was an overcompensation in the daily increase in plant height, and a partial compensation was observed in the 10 d drought stress treatment. For the plant height, the compensation
effect under moderate stress was greater than that under the severe stress treatment, while in the 10 d stress treatment, it was greater than it was in the 5 d stress treatment. The partial compensation of the leaf area was observed in all the treatments after rehydration. The partial compensation in the chlorophyll content of each treatment at the V3 stage followed by rehydration, the effect of drought stress at the V3 stage caused a relatively high level of damage in the chlorophyll. After drought stress at the V3 stage and rehydration, the effect on the RWC could be rapidly reversed. The compensation effect from rehydration after drought stress at the R5 stage was not significant, indicating that drought stress at the V3 stage caused a relatively high level of damage in the chlorophyll. After drought stress at the V3 stage and rehydration, the effect on the RWC could be reversed. In addition, drought hardening at the V3 stage could improve the plant’s ability to recover upon rehydration after drought stress at the R5 stage.

3.2. The effect of drought stress and rehydration on physiological parameters in soybean leaves

Chlorophyll is an important component of the pigment-protein complex on the thylakoid membrane, which is crucial for photosynthesis. The chlorophyll content could reflect the level of photosynthesis to some extent and could further affect plant growth. The plant water status directly affects metabolic processes in plants and therefore impacts plant growth. The relative water content (RWC) in leaves is an important parameter of the plant water content and is closely associated with plant drought resistance. Thus, studying the effect of water stress and rehydration on the chlorophyll content and RWC in soybean leaves is helpful for revealing the tolerance level of soybeans to drought stress.

Table 5 shows that applying drought stress at each growth stage reduced the chlorophyll content and RWC in the soybeans. As the drought stress level increased and the stress duration was prolonged, the decrease in these two parameters became more dramatic. There was no significant difference in the degree of decrease among different growth stages. After rehydration, the chlorophyll content and RWC both increased. There was an overcompensation in the chlorophyll content of each treatment at the V3 stage, while a partial compensation effect was observed in each treatment at the R5 and V3R5 stages. For the RWC, an equal compensation was observed after 5 d of drought stress at the V3 stage, while in the 10 d drought stress treatment, there was an overcompensation. Partial compensation was present in each treatment at the R5 stage; equal compensation was observed in each treatment at the V3R5 stage. These results showed that after drought stress at the V3 stage followed by rehydration, the effect of drought stress on chlorophyll could be rapidly reversed. The compensation effect from rehydration after drought stress at the R5 stage was not significant, indicating that drought stress at the V3 stage caused a relatively high level of damage in the chlorophyll. After drought stress at the V3 stage and rehydration, the effect on the RWC could be reversed. In addition, drought hardening at the V3 stage could improve the plant’s ability to recover upon rehydration after drought stress at the R5 stage.

3.3. The effect on drought stress and rehydration on the osmolytes in soybean leaves

The free proline, soluble sugar, and soluble protein contents in the plants are closely related to plant stress resistance. Generally, these substances play a role in osmoprotection and dehydration prevention under drought stress. Through the regulation of their contents, the water potential values in the cells are reduced to maintain turgor pressure, and therefore normal metabolic activi-

### Table 3
Plant height and Daily increase of different treatments.

| Treatment | Plant height of rewater 0–10 d after drought stress (cm) | Daily increase (cm d⁻¹) |
|-----------|----------------------------------------------------------|-------------------------|
|           | 0 d | 5 d | 10 d | 0 d–5 d | 5 d–10 d |
| CK-V3-5   | 24.64Aa | 28.11Aa | 35.00Aa | 0.69 | 1.38 |
| V3S5      | 21.56Ab | 26.01Aa | 34.13Aa | 0.88 | 1.62 |
| V3S5      | 18.11Bb | 20.17Bb | 29.86Bb | 0.41 | 1.67 |
| V3-R5     | 28.11Aa | 35.00Aa | 41.17Aa | 1.38 | 1.70 |
| V3S10     | 24.19Bb | 31.06Bb | 37.64Bb | 1.37 | 1.32 |
| V3S10     | 16.94Cc | 23.84Cc | 31.48Cc | 1.24 | 1.66 |
| V3-R5     | 66.43Ab | 68.41Ab | 69.66Ab | 0.31 | 0.32 |
| R5-R5     | 62.88Cd | 65.11Cc | 68.11Ck | 0.45 | 0.60 |
| CK-R5-10  | 70.28Aa | 71.37Aa | 72.19Aa | 0.31 | 0.16 |
| R5S10     | 65.41Bb | 68.93Bb | 69.94Ab | 0.31 | 0.25 |
| R5S10     | 61.58Cc | 65.96Cc | 68.17Ck | 0.60 | 0.15 |
| CK-R5-5   | 69.23Aa | 70.28Aa | 71.37Aa | 0.31 | 0.32 |
| V3R5S5    | 58.64Bc | 61.70Cd | 64.39Cd | 0.61 | 0.53 |
| V3R5S5    | 54.27Dd | 58.64Dd | 60.34De | 0.87 | 0.34 |
| V3-R5     | 70.28Aa | 71.37Aa | 72.19Aa | 0.31 | 0.16 |
| CK-R5-10  | 58.16Dd | 61.70Dd | 64.39Dd | 0.61 | 0.53 |
| R5R5S5    | 62.88Cc | 65.11Cc | 68.11Cc | 0.31 | 0.27 |
| CK-R5-5   | 54.78Ee | 56.75Ee | 58.49Ee | 0.60 | 0.13 |

Note: 1. Using LSD test, different small letters mean significant difference at 0.05 level, different capital letters mean extreme significant difference at 0.01 level.

### Table 4
Leaf area under different treatments.

| Treatment | Leaf area of rewater 0–10 d after drought stress (cm²) |
|-----------|----------------------------------------------------------|
|           | 0 d | 5 d | 10 d |
| CK-V3-5   | 610.53Aa | 694.37Aa | 822.15Aa |
| V3S5      | 477.99Ab | 586.39Ab | 829.01Aa |
| V3S5      | 374.09Bc | 469.92Bc | 618.05Bb |
| CK-V3-10  | 692.53Aa | 822.15Aa | 975.13Aa |
| V3S10     | 396.43Bb | 702.26Ab | 965.42Aa |
| CK-V3-10  | 339.42Bb | 645.16Ab | 869.51Aa |
| CK-R5-5   | 1303.26Aa | 1225.65Aa | 1168.32Aa |
| R5-R5     | 1005.85Cc | 950.17Bc | 912.72Bb |
| R5S10     | 832.15Cc | 785.66Cc | 725.17Cc |
| CK-R5-10  | 70.28Aa | 116.32Aa | 102.33Aa |
| R5S10     | 945.32Bb | 884.48Bb | 809.63Bb |
| R5S10     | 760.93Bb | 706.17Cc | 655.32Cc |
| CK-R5-5   | 1303.26Aa | 1225.65Aa | 1168.32Aa |
| V3R5S5    | 1061.72Bb | 992.64Bb | 931.85Bb |
| V3R5S5    | 810.36Cc | 765.32Cc | 714.69Cc |
| CK-R5-10  | 1225.65Aa | 1168.32Aa | 1023.35Aa |
| R3-R5S10  | 792.17Bb | 889.35Bb | 826.47Bb |
| R3-R5S10  | 775.42Bc | 729.93Cc | 674.72Cc |
ties are maintained to adapt to the stressful environment. Among these compounds, free proline is considered to be one of the most important indicators of plant drought resistance.

3.3.1. The effect of drought stress at different growth stages and rehydration on proline contents in soybean leaves

Table 6 shows that drought stress at all the tested growth stages caused the proline contents in the leaves to increase. After rehydration, the proline content under each stress treatment all exhibited a decline. Equal compensation was observed under moderate stress at the V3 stage, while partial compensation was observed in the severe stress treatment. Partial compensation was also present in all treatments at the R5 and V3R5 stages. This finding indicated that the impact of drought stress at the V3 stage on the proline content of soybean leaves required a long time to recover, while drought stress at the R5 stage caused an irreversible impact on the proline content in the leaves. Thus, drought stress should be avoided during the R5 stage, especially a long period of drought stress.

3.3.2. The effect of drought stress at different growth stages and rehydration on the leaf soluble sugar content

As shown in Table 6, the drought stress at each tested growth stage caused the soluble sugar content in the leaves to rise. After rehydration, the soluble sugar contents in each stress treatment all decreased rapidly. Equal compensation was observed in the moderate stress treatment at the V3 stage; partial compensation was present in the severe stress treatment. Among the 5 d of stress treatment at the R5 and V3R5 stages, the V3RSS5 treatment exhibited an overcompensation; R5S5 and V3RSS5 exhibited equal compensation, while R5SS5 exhibited a partial compensation. A partial compensation effect was also observed in the 10 d drought stress treatment. These results showed that the plants could recover or reduce the damage from drought stress by osmoregulation, but the plants could not fully recover from severe-level drought stress at the V3 stage or long-term drought stress at the R5 stage.

3.3.3. The effect of drought stress at different growth stages and rehydration on the soluble protein content in the leaves

The results in Table 6 show that 5 d of drought stress at the V3 stage reduced the soluble protein content in the leaves, and the reduction became more dramatic as the drought stress became more severe. The 10 d stress treatment caused the leaf soluble protein content to increase. The 5 d stress treatment at the R5 and V3R5 stages exhibited an increase in the leaf soluble protein content; the degree of the increase became more dramatic as the drought stress became more severe or less frequent. The 10 d stress

Table 6
Proline content, Soluble sugar content and Soluble protein content in different treatments.

| Treatment | Proline content (µg g⁻¹) | Soluble sugar content (µmol·g⁻¹) | Soluble protein content (µmol·g⁻¹) |
|-----------|-------------------------|----------------------------------|-----------------------------------|
|           | 0 d  | 5 d  | 10 d | 0 d  | 5 d  | 10 d | 0 d  | 5 d  | 10 d |
| CK-V3-5   | 220.17Cc | 242.67Cc | 230.45Ab | 76.25Cc | 77.53Cc | 77.09Bb | 13.79Aa | 13.48Aa | 14.22Aa |
| V3S5     | 323.43Bb | 306.52Bb | 236.34Ab | 148.58Bb | 99.26Bb | 80.97Bb | 13.61Aa | 14.82Aa | 15.70Aa |
| V3SSS5   | 434.84Aa | 354.76Ab | 254.58Aa | 166.63Aa | 124.38Aa | 110.22Aa | 11.98Aa | 13.76Aa | 13.75Aa |
| V3R5     | 242.67Cc | 230.45Cc | 237.82Cbc | 77.53Cc | 77.09Cc | 76.22Ab | 13.48Aa | 14.22Aa | 12.58Aa |
| V3S5S5   | 465.48Bb | 328.19Bb | 256.18Bb | 160.23Bb | 89.56Aa | 77.37Ab | 15.17Aa | 13.83Aa | 11.58Aa |
| CK-R5-10 | 46.37Aa | 46.37Aa | 46.49Aa | 46.40Aa | 46.40Aa | 46.40Aa | 46.40Aa | 46.40Aa | 46.40Aa |
| R5S5     | 39.69Cc | 40.59Cc | 42.35Cc | 48.21Cc | 62.62Ab | 64.88Aa | 69.29Aa | 69.69Aa | 69.69Aa |
| R5SS5    | 43.26Bb | 44.44Bb | 44.42Bb | 62.62Ab | 64.88Aa | 69.29Aa | 69.69Aa | 69.69Aa | 69.69Aa |
| CK-V3-10 | 242.67Cc | 245.92Cc | 245.92Cc | 144.11Cc | 152.25Cb | 152.50Bc | 17.02Aa | 17.79Aa | 17.23Aa |
| V3SS10   | 306.22Cc | 306.22Cc | 306.22Cc | 144.11Cc | 152.25Cb | 152.50Bc | 17.02Aa | 17.79Aa | 17.23Aa |
| V3S5S10  | 38.02Cc | 39.10Cc | 41.01Cc | 42.84Cc | 66.13Ab | 66.34Ab | 66.34Ab | 66.34Ab | 66.34Ab |
| V3R5S10  | 41.03Bb | 42.31Bb | 42.91Bb | 57.39Bb | 62.71Bc | 64.54Bb | 64.54Bb | 64.54Bb | 64.54Bb |
| R5S10    | 41.03Bb | 42.31Bb | 42.91Bb | 57.39Bb | 62.71Bc | 64.54Bb | 64.54Bb | 64.54Bb | 64.54Bb |
| R5SS10   | 41.03Bb | 42.31Bb | 42.91Bb | 57.39Bb | 62.71Bc | 64.54Bb | 64.54Bb | 64.54Bb | 64.54Bb |
| V3SSS10  | 561.95Aa | 425.55Aa | 306.24Aa | 185.58Aa | 83.16Ab | 83.16Ab | 12.93Aa | 12.93Aa | 12.93Aa |
| V3S10    | 465.54Bb | 328.19Bb | 256.18Bb | 160.23Bb | 89.56Aa | 77.37Ab | 15.17Aa | 13.83Aa | 11.58Aa |
| V3R5S10  | 465.54Bb | 328.19Bb | 256.18Bb | 160.23Bb | 89.56Aa | 77.37Ab | 15.17Aa | 13.83Aa | 11.58Aa |
| V3SSS5   | 397.90dd | 344.00dd | 307.52Aa | 217.81Cd | 196.30Cd | 167.69Bc | 16.31Aab | 10.86Ccd | 14.77Bb |
| V3SS10   | 327.14Cc | 279.18Cc | 246.27Cc | 175.12Bd | 154.10Cb | 138.00Cc | 17.74Aa | 15.74Ab | 15.47Ab |
| V3RSS5   | 327.14Cc | 279.18Cc | 246.27Cc | 175.12Bd | 154.10Cb | 138.00Cc | 17.74Aa | 15.74Ab | 15.47Ab |
| V3RSS10  | 366.06Bb | 328.19Bb | 256.18Bb | 160.23Bb | 89.56Aa | 77.37Ab | 15.17Aa | 13.83Aa | 11.58Aa |
| V3RSSS10 | 465.54Bb | 328.19Bb | 256.18Bb | 160.23Bb | 89.56Aa | 77.37Ab | 15.17Aa | 13.83Aa | 11.58Aa |

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treatment reduced the soluble protein content, and this decrease became more dramatic as the drought stress became more severe and less frequent. After rehydration, the 5 d stress treatment at the V3 stage exhibited an increase in the soluble protein content, while the 10 d stress treatment exhibited a decrease. Each 5 d stress treatment at the R5 and V3RS stages led to a decrease in the soluble protein content. Each stress treatment involving 10 d of drought stress first showed a decrease and then an increase in the soluble protein content. Each V3 stage treatment exhibited equal compensation, while injuries were present in all the treatments at the R5 and V3RS stages. This result suggested that drought stress at the V3 stage did not significantly affect the soluble protein content in the leaves, but drought stress at the R5 stage resulted in injuries beyond recovery after rehydration.

3.4. The effect of drought stress and rehydration on antioxidant enzymes and membrane lipid peroxidation in soybean leaves

Under normal conditions, antioxidant enzymes have relatively high activities so that they can effectively remove ROS from plants. Many studies have shown that under drought stress and rehydration, antioxidant enzyme activities undergo major changes; these changes differ depending on the experimental materials, the type of antioxidant enzyme, the stress level and duration, etc. Usually, the entire antioxidant system needs to be activated in plants to remove excessive ROS effectively.

The cell membrane is the most sensitive organelle to drought stress; the level of damage in the crop cell membrane from drought varies depends on the varieties, the level and the timing of the stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress; the level of damage in the crop cell membrane from drought stress. The plasma membrane is the primary location damaged after long-term drought stress. Soybeans at the R5 stage could adapt to short-term moderate drought stress, but long-term medium and severe drought stress could damage the membrane lipid peroxidation. The content of the final product, malondialdehyde (MDA), will also increase. In addition, drought stress leads to cell membrane damage; an increase in membrane permeability causes the cell to lose control over its content and increase the electrical conductance due to electrolyte leakage. Thus, the permeability of the plasma membrane has become a parameter of drought stress resistance. Because the MDA content can reflect the degree of membrane lipid peroxidation in the leaves, it is also used as a parameter for indicating drought stress resistance.

3.4.1. The effect of drought stress at different growth stages and rehydration on the activity of superoxide dismutase (SOD) in the leaves

As shown in Table 7, moderate drought stress at the V3 stage induced SOD activity, and this increase slowed down as the drought stress was prolonged. A 5 d treatment with severe drought stress also induced SOD activity, but 10 d of severe stress reduced it. The 5 d treatment applying moderate drought stress at the R5 stage caused the SOD activity to increase in the leaves, while 5 d of severe stress and 10 d of moderate stress reduced it. In addition, the rate of decrease in the SOD activity accelerated as the stress duration was prolonged and became more severe, and the rate slowed down as the stress frequency increased. After rehydration, the SOD activity in the 5 d drought stress treatment at the V3 stage decreased rapidly and exhibited equal compensation. The V3S10 treatment also exhibited equal compensation, while partial compensation was present in the V3SS10 treatment. Each treatment at the R5 and V3RS stages all exhibited partial compensation. This finding indicated that moderate drought stress at the V3 stage and short-term severe drought stress could induce SOD activity in the leaves. However, long-term severe stress could inhibit SOD activity. Soybeans at the R5 stage could adapt to short-term moderate drought stress, but long-term medium and severe drought stress could damage the SOD activity in soybean leaves.

3.4.2. The effect of drought stress at different growth stages and rehydration on the peroxidase (POD) activity in the leaves

As shown in Table 7, drought stress at the V3 stage induced POD activity in the leaves; the rate of increase became more dramatic as the stress level was elevated. A longer moderate stress treatment caused the increase rate in the POD activity to rise, while under severe stress, this increase rate declined. A 5 d moderate drought stress treatment at the R5 stage increased the POD activity in the leaves; 5 days of severe stress and 10 d of drought stress could cause the POD activity to decrease, with the rate declining as the stress duration was prolonged. After rehydration, the 5 d drought stress treatment at the V3 stage exhibited equal compensation, while the 10 d drought stress treatment exhibited partial compensation. All the treatments at the R5 and V3RS stages exhibited equal compensation. These results showed that drought stress at the V3 stage could induce POD activity in the leaves. After drought stress and rehydration, the impact caused by short-term drought stress on the POD activity could recover, but it could only partially recover after long-term drought stress. Soybeans at the R5 stage could

Table 7

| Treatment       | SOD activity (U g⁻¹FW min⁻¹) | POD activity (U g⁻¹FW min⁻¹) | CAT activity (U g⁻¹FW min⁻¹) | Membership value |
|-----------------|-------------------------------|-------------------------------|-------------------------------|------------------|
|                 | 0 d                           | 5 d                           | 10 d                          |                  |
| CK-V3-5         | 545.82Cc                      | 579.38Bb                      | 553.29Aa                      |                  |
| V3SS            | 769.23Aa                      | 622.18Aa                      | 549.17Aa                      |                  |
| V3SSS           | 651.17Bb                      | 604.32Ab                      | 535.60Bb                      |                  |
| CK-V3-10        | 579.38Bb                      | 553.29Bb                      | 550.81Aa                      |                  |
| V35T            | 677.47Aa                      | 589.53Aa                      | 535.60Bb                      |                  |
| V3S10           | 465.30Cc                      | 488.52Cc                      | 516.75Bb                      |                  |
| CK-R5-5         | 470.50Cc                      | 478.01Bb                      | 426.66Cg                      |                  |
| R5SS            | 574.72Aa                      | 518.93Aa                      | 444.36Aa                      |                  |
| R5SSS           | 418.35 dd                     | 385.97Ccc                     | 402.18Bdd                     |                  |
| CK-R5-10        | 478.01Aa                      | 426.66Aa                      | 437.22Aa                      |                  |
| R5S10           | 433.69Bc                      | 409.86ABab                    | 425.76ABab                    |                  |
| V3SSS           | 651.17Bb                      | 604.32Ab                      | 535.60Bb                      |                  |
| V3R5-10         | 520.98Bb                      | 508.72Ab                      | 436.15Ab                      |                  |
| V3R5SS          | 455.65 dd                     | 395.11Cc                      | 410.98Bcd                     |                  |
| R5V3S10         | 478.01Aa                      | 426.66Aa                      | 437.22Aa                      |                  |
| R5V3SSS         | 373.69Cdc                     | 391.06Bdc                     | 403.02Cddc                    |                  |
adapt to short-term moderate drought stress, but long-term and severe stress would injure the leaf POD activity. The effect of drought stress at the R5 stage on the POD activity in soybean leaves could be returned to a normal level shortly after rehydration.

3.4.3 The effect of drought stress at different growth stages on the CAT activity in the leaves

Table 7 shows that different drought stress levels had different effects on the CAT activity in soybean leaves. Moderate stress at the V3 stage induced CAT activity; the rate of increase accelerated as the stress duration was prolonged. Severe stress reduced the CAT activity, and the rate of decrease became more dramatic as the stress duration was prolonged. Drought stress at the R5 stage reduced the CAT activity and the rate of decrease accelerated with a more severe stress level and a longer duration. After rehydration, equal compensation was observed under moderate stress at the V3 stage, while severe stress treatment yielded overcompensation. For treatments at R5 and V3R5, the 5 d moderate stress treatment exhibited equal compensation; 10 d treatments with moderate and severe stress exhibited partial compensation. This finding showed that moderate stress at the V3 stage induced CAT activity, which was significantly inhibited by severe stress. Drought stress at the R5 stage caused damage to the CAT activity, and drought resistance at the V3 stage could weaken this damage. After drought stress at the R5 stage and rehydration, the plants could recover from the damage in leaf CAT activity by short-term moderate stress, while only partial recovery from the damage caused by long-term drought stress and severe stress was observed.

3.4.4 Comprehensive evaluation of the drought stress effects at different growth stages on the antioxidant potential in leaves

As shown in Table 7, drought stress at the V3 stage could induce the antioxidant potential in leaves. As the level of drought stress became more severe, the antioxidant potential decreased in all the stress treatments. The 5 d moderate stress treatment increased the antioxidant potential at the R5 stage, which was reduced by 5 d of severe stress and 10 d of drought stress. This decreasing trend became more dramatic with more severe drought stress levels. The antioxidant potential was decreased to almost zero in severe stress treatments. After rehydration, the antioxidant potential decreased with time in the 5 d drought stress treatment at the V3 stage and the 10 d moderate stress treatment. However, the 10 d treatment with severe stress exhibited an increase in the antioxidant potential. All the treatments also exhibited an increasing trend in the antioxidant potential at the R5 stage. This result indicated that after drought stress at the V3 stage and rehydration, the soybean leaves could recover from the damage in the protective enzyme system caused by drought stress, but they could not recover from the damage caused by drought stress at the R5 stage. The antioxidant protective system had a relatively high ability to adapt to a drought stress duration of shorter than 10 d at the V3 stage; drought stress at the R5 stage should thus be avoided.

3.4.5 The effect of drought stress at different growth stages and rehydration on the permeability of the plasma membrane

Table 8 shows that drought stress at all growth stages increased the relative electrical conductivity of leaves; the rate of increase accelerated as the drought stress became more severe and lasted longer. After rehydration, the 5 d drought stress treatment yielded equal compensation at the V3 stage, while the 10 d drought stress treatment exhibited partial compensation. Equal compensation was observed in all stress treatments at the R5 stage. This finding indicated that upon rehydration after short-term drought stress at the V3 stage, the effect of drought stress on the relative electrical conductivity in soybean leaves could be recovered, but it could only be partially recovered after long-term drought stress, or it would take more time to recover. Upon rehydration after drought stress at the R5 stage, the effect of the stress on the relative electrical conductivity in leaves could be restored.

3.4.6 The effect of drought stress at different growth stages and rehydration on the leaf MDA content

As shown in Table 8, the leaf MDA content increased in the drought stress treatments at all the growth stages, and the rate of this increase accelerated with more severe and longer lasting drought stress. After rehydration, 5 d of drought stress yielded equal compensation at the V3 stage, while the 10 d drought stress treatment yielded overcompensation. The 5 d drought stress treatment exhibited equal compensation at the R5 stage. All 10 d drought stress treatments exhibited partial compensation at the R5 stage, while all treatments exhibited equal compensation at V3R5. These results indicated that drought stress caused damage to the plasma membrane of the soybean leaves, and the damage was greater when the drought occurred during later growth stages than earlier ones. Drought during the V3 stage could increase the adaptivity to drought stress during later growth stages. Upon rehydration after drought stress at the V3 stage, the membrane lipid peroxidation caused by the drought stress could be restored, while

| Treatment | Leaf relative conductance (%) | MDA content (nmol g⁻¹) |
|-----------|--------------------------------|------------------------|
|           | 0 d                            | 5 d                    | 10 d                   |
|           | 0 d                            | 5 d                    | 10 d                   |
| CK-V3-5   | 0.31Bb 0.32Ab 0.31Aa           | 29.98Bb 29.93Aa 29.63Aa |
| V3SS      | 0.35AAb 0.34Aab 0.29Ab         | 31.95Ab 29.43Aa 28.13Aa |
| V3SSS     | 0.39Aa 0.35Aa 0.31Aa           | 33.17Aa 30.17Aa 27.80Aa |
| CK-V3-10  | 0.32Cc 0.31Bb 0.33Aa           | 29.93Bc 29.63Aa 29.63Aa |
| V3S10     | 0.38Bb 0.35Ab 0.34Aa           | 34.85Ab 30.03Aa 24.85Ab |
| V3SS10    | 0.44Aa 0.37Aa 0.33Aa           | 36.76Aa 28.38Aa 25.11Ab |
| CK-R5-5   | 0.34Cc 0.33Cc 0.34Cc           | 56.93Dd 56.49Cd 54.36Bb |
| R5SS      | 0.38BCb 0.35Bcc 0.35Aa         | 122.68BCb 98.63Ab 64.19Ab |
| R5SSS     | 0.41AAb 0.37Ab 0.33Aa         | 159.83Aa 104.86Aa 72.46Aa |
| CK-R5-10  | 0.33Dc 0.34Bc 0.33Aa           | 56.49Dd 54.36Dd 57.11Cdd |
| R5S10     | 0.42Cbc 0.37Bcab 0.35Aa        | 182.63Bb 145.54Aa 86.57Bb |
| R5SS10    | 0.46Aa 0.40Aa 0.36Aa           | 213.09Aa 160.54Aa 107.03Aa |
| CK-R5-5   | 0.34Cc 0.33Cc 0.34Cc           | 56.93Dd 56.49Cd 54.36Bb |
| V3R5S5    | 0.39Ab 0.34Abc 0.33Aa         | 98.32Cc 75.93Cc 53.29Bb |
| V3R5SS5   | 0.44Aa 0.39Aa 0.36Aa           | 134.09Ab 82.86Ab 61.41Ab |
| CK-R5-10  | 0.33Dc 0.34Bc 0.33Aa           | 56.49Dd 54.36Dd 57.11Cdd |
| R5S10     | 0.41Cb 0.36Abc 0.35Aa         | 140.82Cc 87.51Cc 52.29Dd |
| V3R5SS10  | 0.45Ab 0.39Aa 0.34Aa           | 176.91Bb 112.24Bb 68.92Cc |
| V3R5SS10  | 0.45Ab 0.39Aa 0.34Aa           | 176.91Bb 112.24Bb 68.92Cc |

Table 8 Leaf relative conductance and MDA content in different treatments.
3.5. The effect of drought stress and rehydration on endogenous hormones in soybean leaves

Drought stress induces a series of changes in plant endogenous hormones and their ratios, representing plant adaptations to water stress. Drought resistance in plants is not achieved by a single hormone, but rather through complex coordination among the various involved hormones. Among them, abscisic acid (ABA), which exhibits the most significant changes among the plant endogenous hormones, plays crucial regulatory roles in plant growth and development as well as stress resistance. Upon drought stress, an important physiological function of ABA is to reduce the number of ions in the guard cells to decrease the turgor pressure, which induces stomatal closure. In addition, ABA can reduce plant water consumption and enhance the water retention capacity under water deficiency. Thus, ABA is often used as an important parameter to assess plant drought resistance. Auxin (IAA) is a plant hormone that promotes growth and is synthesized in the apical meristems and growing leaves. When plants encounter drought, their growth is inhibited, leading to a decrease in the IAA-hormone system. Therefore, there is an interactive balance between IAA and growing tissues. Zeatin riboside (ZR) is the most common form of cytokinin in plants, and it promotes cell division and expansion and lateral bud development. ZR also delays senescence and facilitates the transport of nutrients. Gibberellic acid (GA) is a growth-promoting compound related to flower formation and internode elongation. As shown in Table 9, on 0 d stress of rehydration after drought stress, the ABA content in the leaves increased, while the GA and ZR contents decreased. Their content was positively correlated with more severe and longer lasting drought stress. The IAA content decreased in the treatments at the V3 stage, while it increased in the treatments at the R5 and V3R5 stages. At 10 d after rehydration, all the hormones in each stress treatment either increased or decreased to levels that were not significant to the CK; equal compensation or nearly equal compensation was observed. Drought stress reduced the ZR/IAA, ZR/ABA, and ABA/[IAA + GA + ZR] ratios (Table 10), indicating that the antagonism among endogenous hormones during drought stress inhibited leaf differentiation and induced stomatal closure to conserve water; plant growth was inhibited. Rehydration yielded equal compensation or nearly equal compensation, as represented by the promotion of leaf differentiation, stomatal opening, and photosynthesis, and plant growth was induced.

4. Discussion

Plant growth compensation upon rehydration after drought stress occurs throughout the entire life cycle as a possible plant self-defence against short-term, periodic, or unpredictable drought. Acevedo et al. (1971) demonstrated short, rapid growth upon rehydration after water stress as a compensation, or partial compensation, for the losses caused by water stress, similar to our results in this study. Chen et al. (2001) found that in winter wheat, a drought during the seedling and booting stages followed by rehydration at the jointing and heading stages led to significant growth compensation in terms of the plant height and leaf area. Additionally, rehydration after moderate drought stress led to overcompensation, which was different from the results in this study. This result was different from that in this study, in which the compensation effect in plants challenged with drought stress two times was smaller than it was in plants that were treated with drought stress only once. The results in this study showed that after drought stress and rehydration, the soybean plant height and leaf area grew faster, reducing the difference from the control and indicated growth compensation. The compensation effect was dependent on the level of drought stress and the growth stage when the drought stress occurred as well as the duration and frequency of the drought stress. In addition, our results indicated that the growth inhibition by drought stress during each growth stage tested here could not be completely compensated after rehydration. A milder level or shorter and less frequent water stress resulted in greater compensation. In addition, the compensation effect was greater if stress occurred at early growth stages compared to later growth stages.

Many studies have shown that drought stress and rehydration could trigger a series of physio-biochemical compensation in crops. Bielorai and Hopmans (1975), Xu and Bland (1993) found that in cotton and sorghum, the leaf water potential recovered rapidly, at 2–3 days after drought stress and rehydration, consistent with the results in this study. Studies by Zhang et al. in soybeans and wheat showed that after drought stress and rehydration, plants maintained high levels of SOD, APX, and CAT activity to remove ROS effectively and mitigate membrane damage and membrane lipid peroxidation, consistent with the results in this study.

| Treatment         | ABA content (ng/g DW) | GA content (ng/g DW) | IAA content (ng/g DW) | ZR content (ng/g DW) |
|-------------------|-----------------------|----------------------|-----------------------|----------------------|
| CK-V3-5           | 73.56Cc               | 69.62Bh              | 74.88Bb               | 21.97Aa              |
| V3S5              | 88.11Bb               | 75.68Aa              | 78.15Aa               | 17.87Aa              |
| V3S5              | 93.75Aa               | 78.65Aa              | 78.4Aa                | 15.34Cc              |
| CK-V3-10          | 69.62Cc               | 74.88Bb              | 69.39Aa               | 24.04Aa              |
| V3S10             | 90.67Bb               | 81.28Aa              | 74.77Aa               | 15.85Bb              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
| CK-R5-5           | 92.76B38C            | 89.840C              | 87.14Aa               | 36.04Aa              |
| RS5S              | 105.51Aab             | 94.837B              | 82.64Aa               | 33.21Aa              |
| RS5S              | 109.52Aa              | 101.04Aa             | 83.04Aa               | 26.26Aa              |
| CK-R5-10          | 89.840Bb              | 82.47Cc              | 91.92Aa               | 38.59Aa              |
| RS5S              | 112.38Aa              | 96.67Bb              | 85.98Aa               | 29.86Bb              |
| RS5S              | 123.97Aa              | 101.25Ab             | 93.19Aa               | 23.07Cc              |
| CK-R5-5           | 92.76B38C            | 89.840C              | 87.14Aa               | 36.04Aa              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
| CK-R5-5           | 92.76B38C            | 89.840C              | 87.14Aa               | 36.04Aa              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
| CK-R5-5           | 92.76B38C            | 89.840C              | 87.14Aa               | 36.04Aa              |
| V3S10             | 105.97Aa              | 78.84Aa              | 68.85Aa               | 12.58Cc              |
Stress at the R5 stage and rehydration only led to slight compensation. This finding is consistent with our results in which drought stress at the seedling and early flowering stages followed by rehydration, the SOD, POD, MDA, and membrane permeability decreased. Partial compensation was observed in the POD activity and membrane permeability at the V3 stage; overcompensation was observed in the MDA content. This overcompensation was more significant when the stress level became more severe and the duration became longer. Equal compensation was observed in all the other treatments. After drought stress and rehydration at the V3 stage, the CAT activity decreased, and then it increased after drought stress at the R5 stage and rehydration; partial compensation was observed in stress treatments at the V3 and R5 stages. The activity of each protective enzyme tested here had better recovery in the stress treatment at the V3R5 stage than the R5. Because the protective enzyme activity recovered after stress at the V3 stage and rehydration, the antioxidant potential decreased, but it increased in the stress treatment at the R5 stage. This finding may be from plants undergoing senescence at later growth stages. For endogenous hormones, after drought stress at each growth stage and rehydration, the ABA content and ABA/(IAA + GA + ZR) decreased. The GA, ZR content, ZR/IAA, and ZR/ABA increased. After drought stress at the V3 stage and rehydration, the antioxidant potential decreased, but it increased in the stress treatment at the R5 stage. This finding may be from plants undergoing senescence at later growth stages. For endogenous hormones, after drought stress at each growth stage and rehydration, the ABA content and ABA/(IAA + GA + ZR) decreased. The GA, ZR content, ZR/IAA, and ZR/ABA increased. After drought stress at the V3 stage and rehydration, the antioxidant potential decreased, but it increased in the stress treatment at the R5 stage. This finding may be from plants undergoing senescence at later growth stages. For endogenous hormones, after drought stress at each growth stage and rehydration, the ABA content and ABA/(IAA + GA + ZR) decreased. The GA, ZR content, ZR/IAA, and ZR/ABA increased. After drought stress at the V3 stage and rehydration, the antioxidant potential decreased, but it increased in the stress treatment at the R5 stage. This finding may be from plants undergoing senescence at later growth stages.

5. Conclusion

Drought stress inhibited increases in the soybean plant height and leaf area. As the stress became more severe, longer, and more frequent, this inhibitory effect became more significant. After rehydration, the soybean plant height and leaf area exhibited a rapid increase and produced partial compensation. As the level, duration, and frequency of drought stress increased, the compensation effect decreased, but it did not return to the control level.
Drought stress reduced the chlorophyll content and relative water content in soybean leaves. Drought stress also increased the contents of osmolytes, and it enhanced the antioxidant potential and peroxidation of membrane lipids. In addition, these changes mentioned above became more dramatic as the drought stress level, duration, and frequency increased. Upon rehydration, various levels of compensation were observed in each physio-biochemical parameter. As the drought stress level, duration, and frequency increased, the compensation effect also increased. Overall, the compensation effect for drought stress that occurred at the early growth stages was higher than that at the later growth stages.

Drought stress led to a decrease in the ZR/IAA and ZR/ABA ratios in soybean leaves and an increase in the ABA/(IAA + GA + ZR) ratio, and plant growth was inhibited. These hormone ratios exhibited more dramatic changes when the drought stress level became more severe and the duration was prolonged. After rehydration, these hormone ratios exhibited equal compensation.

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