# BENEFICIAL EFFECTS OF MILD WATER DEFICIENCY ON GROWTH AND YIELD IN THE SEEDLING STAGE OF SOYBEAN

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(Received 11th Apr 2024; accepted 25th Sep 2024)

**Abstract.** This experiment was conducted at Heilongjiang Bayi Agricultural University in China, using Sui Nong 26 soybeans and negative pressure water supply equipment. The study set different water control periods (V1, R1, and R5 stages) and varying degrees of water stress (normal, mild, moderate, severe) to analyze soybean yield, nutrient uptake, growth, and physiological and biochemical responses to drought and water compensation. The aim was to establish high-quality, high-yield, water-saving cultivation techniques for dryland soybeans. Results showed that increasing water stress decreased soybean plant height and stem diameter. At the V1 and R1 stages, root length initially decreased and then increased, while drought stress at R1 and R5 stages inhibited dry matter accumulation. Severe drought stress significantly reduced dry matter compared to mild stress. Mild drought stress at the seedling stage increased dry matter accumulation, but severe stress hindered recovery after rehydration. During the V1 stage, mild drought stress increased Pn, Tr, and Gs by 7.9%, 16.4%, and 1.0%, respectively. In the R1 stage, drought stress reduced Pn, Gs, Ci, and Tr, with greater reductions under more severe stress. Post-rehydration, Pn, Gs, and Ci under mild stress were higher than CK. At the R5 stage, rehydration failed to restore photosynthetic capacity. Mild drought stress increased grain yield by 5.0% at the seedling stage but reduced it during flowering and bulging stages, also increasing grain protein content while decreasing lipid content.
**Keywords:** *soybean, drought stress, physiological characteristics, growing development, yield*

## Introduction

Soybean (*Glycine max* L.), is one of our country's main food crops (Zhu et al., 2008). Its nutrient rich seeds are the main source of nutrients for human beings, as well as the main source of feed for various animals. In recent years, the demand for soybeans in China has been increasing annually. Although domestic production has increased, the volume of imports still exceeds domestic output by more than five times (Li et al., 2008). Soybean is a kind of legume crop which is sensitive to water requirement. The drought and the shortage of water affect its growth and development, physiological and biochemical metabolism, and ultimately lead to the reduction of yield and quality (Cui et al., 2012).

By 2050, the world's population is expected to reach 9.8 billion, and the demand for food crops is continuously increasing (Gebre and Earl, 2021). In recent years, global climate change has led to intensified droughts, posing a severe threat to food production. One-third of the world's arable land is in a state of water scarcity, making water shortage a global issue limiting agricultural production. Poudel et al. (2023) recent study indicates that ongoing climate changes, characterized by unstable rainfall, pose a serious threat to food security and raise concerns about their impact on the food chain. In many regions of the world, including the United States, drought stress has become one of the key factors affecting soybean stability and productivity (Poudel et al., 2023). Gebre and Earl (2021) recent research findings show that in most growing seasons, soil moisture deficit


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_215234

constitutes a significant limitation to soybean yield in Ontario, Canada. Even in unusually wet years, soybean yields in Ontario are reduced due to transient soil water deficits, and in drought years, yield losses may exceed 25% (Gebre and Earl, 2021).

China is a big agricultural production country. As the main soybean production area in China, Heilongjiang province is also the place where meteorological disasters frequently occur in China, mainly spring drought, which has gradually aggravated in recent years, resulting in huge losses. At the same time, the low agricultural water utilization efficiency is also a serious problem in Chinese agricultural production, which severely restricts Chinese agricultural development (Li et al., 2003, 2019; Chen et al., 2009; Fan et al., 2019; Zhang et al., 2020) How to improve the water utilization rate of soybean under the situation of agricultural water shortage, ensure the normal growth and development of soybean, increase production and sustainable development of agriculture is the primary purpose of the study, and the only way for agricultural development in our country. Therefore, it is of great scientific significance to study the effects of drought rehydration on crop growth, yield and nutrient utilization in agricultural production (Xu et al.,1989; Li, 2019).

Currently, the prevalent method for controlling water during drought stress is the weighing method. Its water control period is single. By contrast, this experiment uses the negative pressure water control device to supply water more accurately. This experiment focused on the soybean variety Sui Nong 26, conducting water control treatments during several key growth stages. The study aimed to investigate the effects of varying degrees of water control on the physiological and biochemical metabolism, yield, and quality of soybeans. The goal was to elucidate the mechanisms of drought stress impact and the compensation mechanisms under drought stress, providing a theoretical basis for timely irrigation and drought resistance measures in soybean production.

## Materials and methods

### *Test material*

The experiment was conducted from 2019 to 2020 in a rainproof plastic greenhouse at the experimental base of the National Engineering and Technology Research Center for Coarse Grains, located at Heilongjiang Bayi Agricultural University in China. The tested variety is Suinong 26. The soil used for testing was collected from the topsoil layer (0-20 cm) of the experimental base adjacent to Heilongjiang Bayi Agricultural University. Basic soil fertility is as follows: total nitrogen 1.29 g/kg, total phosphorus 0.48 g/kg, alkali-hydrolyzed nitrogen 90.68 mg/kg, available phosphorus 44.15 mg/kg, available potassium 120 mg/kg, organic matter 22.99 g/kg. The negative pressure irrigation device used in this experiment was developed by the Institute of Agricultural Resources and Agricultural Regionalization, Chinese Academy of Agricultural Sciences. The device is mainly composed of water outlet, water storage bucket and negative pressure stabilizer. It is connected to a type of clay pipe that is permeable to water but airtight".

According to the negative pressure permeation principle, the difference between the soil water potential and the water supply pressure of the irrigation system is used as the driving force for water to enter the crop rhizosphere soil, so as to realize an irrigation method of replenishing water to the crop root soil. *Figure 1* shows the negative pressure irrigation device.


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***Figure 1.*** *Installation diagram (a) Water outlet: Connects to the clay pipe, controlling water entry into the root zone (b) Water storage bucket: Stores water and maintains its level (c) Negative pressure stabilizer: Ensures a stable water supply by regulating negative pressure (d) Clay pipe: Buried in the root zone, allows water infiltration based on negative pressure*

### *Experimental design*

Four water control levels are set for this test: Compared with CK (Weighting method was used to control 80% of soil field moisture capacity), T1 (-5 kpa, 70% of field moisture capacity), T2 (-10kPa, 60% of field moisture capacity), T3 (-15 kpa, 50% of field moisture capacity). The amount of fertilizer is as follows: N: 150mg/kg soil; $P_2O_5$: 100 mg/kg soil; $K_2O$: 150 mg/kg soil. Mix the fertilizer with the sifted soil in a pot. A completely randomized experimental design was adopted, with 6 pots sampled for each treatment and 6 pots reserved for each treatment for yield measurement. A total of 204 pots were sown on May 26. There were 3 plants per pot in V1 stage. Water control was carried out at V1 stage, R1 stage and R5 stage of soybean. Rehydration shall be conducted after 15 days of water control in each stage, and plant samples shall be taken for test after 15 days of water control and 7 days of rehydration. CK treatment potted plants with 5cm drying topsoil as the limit, artificial watering. The specific soil physicochemical properties and treatment methods are shown in *Table 1* and *Table 2*.

***Table 1.*** *The soil physicochemical properties*

| Soil Property | Value |
|---|---|
| Total Nitrogen | 1.29 g/kg |
| Total Phosphorus | 0.48 g/kg |
| Alkali-hydrolyzable Nitrogen | 90.68 mg/kg |
| Available Phosphorus | 44.15 mg/kg |
| Available Potassium | 120 mg/kg |
| Organic Matter | 22.99 g/kg |

### *Experimental background*

In recent years, significant research has focused on understanding the various growth stages of soybeans, specifically V1, R1, and R5, to improve agricultural practices and productivity.


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***Table 2.** Experimental design*

| Treatments | CK | V1-T1 | V1-T2 | V1-T3 | R1-T1 | R1-T2 | R1-T3 | R5-T1 | R5-T2 | R5-T3 |
|---|---|---|---|---|---|---|---|---|---|---|
| Lower limit of soil water potential | CK | -5kPa | -10kPa | -15kPa | -5kPa | -10kPa | -15kPa | -5kPa | -10kPa | -15kPa |
| Water control period | — | V1 phase water control for 15 days | V1 phase water control for 15 days | V1 phase water control for 15 days | — | — | — | — | — | — |
| | — | — | — | — | R1 phase water control for 15 days | R1 phase water control for 15 days | R1 phase water control for 15 days | — | — | — |
| | — | — | — | — | — | — | — | R5 phase water control for 15 days | R5 phase water control for 15 days | R5 phase water control for 15 days |

The V1 stage marks the first fully expanded trifoliolate leaf and is crucial for establishing vegetative growth. Early phenotyping studies have highlighted the importance of root traits at this stage for drought tolerance and overall plant health (Wang et al., 2021; El-Batal et al., 2020). The R1 stage signifies the beginning of flowering, which is critical for transitioning from vegetative to reproductive growth. Studies have shown that the microbial community in the rhizosphere changes significantly at this stage, impacting nitrogen metabolism and plant health (Ghizzi et al., 2020). The impact of environmental factors like day length and temperature on the flowering stage has been well-documented, showing that early flowering can be a trait selected for improved yield under various climatic conditions (Negrea et al., 2020). The R5 stage is characterized by the beginning of seed fill, which directly affects yield potential (Chennupati et al., 2011). Additionally, the R5 stage is crucial for source-sink dynamics, where increasing assimilate supply to seeds during this phase significantly boosts final seed weight. This highlights the importance of managing assimilate supply to maximize yield (Chiluwal et al., 2021).

Recent studies underscore the importance of accurately defining and understanding soybean growth stages, particularly V1, R1, and R5, for optimizing agricultural practices and improving crop yields.

### *Experimental conditions*

This experiment was conducted from 2019 to 2020 in a rainproof plastic greenhouse at the National Coarse Cereals Engineering Research Center in Daqing, Heilongjiang Province, China. Daqing is located in the western part of Heilongjiang Province and


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experiences a relatively short frost-free period throughout the year. The climate is characterized as a northern cold temperate continental monsoon climate. The rainproof plastic greenhouse provided a controlled environment that protected the plants from external rainfall. The temperature and humidity within the greenhouse were maintained at stable levels to ensure consistent growth conditions for the plants.

### *Plant protection measures*

Integrated pest management practices were employed to protect the plants from pests and diseases. Regular monitoring and appropriate interventions ensured the health and growth of the plants throughout the experimental period.

### *Test items and methods*

Samples were taken at 9:00 am, and plant samples were collected according to the relevant criteria for physiological indicators.

#### *Determination of dry matter*

Soybean plants with uniform growth were selected for each treatment, and the soybean plants were washed with clean water, separated according to different organs, killing at 105℃for 30 minutes, dried at 80℃to constant weight, and then measured by weighing method.

#### *Determination of gas exchange parameters*

Gas exchange parameters were measured using a LI-6400 Photosynthesis System (LI-COR, USA), equipped with an LED red and blue light source leaf chamber and a carbon dioxide gas supply system. Set light intensity of 800μmol·$m^{-2}$·$s^{-1}$, carbon dioxide concentration of 400 μmol·$L^{-1}$ and leaf chamber temperature of 25℃. The sampling time was set from 9:00 AM to 11:00 AM during the V1, R1, and R5 stages to measure parameters such as the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular carbon dioxide concentration (Ci) of the second leaf from the top in fully grown plants under different treatments.

#### *Yield measurement*

Soybean quality index was determined by taking soybean seeds at maturity stage (R8).

#### *Measurement of root activity*

Plants with uniform growth were selected and carefully excavated from the soil. The entire soybean plants were then cut along the cotyledonary node, separating the aboveground and underground parts. The roots (underground part) were thoroughly washed with clean water to obtain intact root systems, and surface moisture was removed using filter paper. This preparation was done to facilitate the measurement of root activity indicators.

Root activity was determined using the TTC (triphenyltetrazolium chloride) reduction method. The activity was quantified based on the amount of triphenylformazan (TTF), a red and water-insoluble compound, produced upon reduction. The quantity of TTF formed indicates the level of root activity.


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### *Determination of leaf antioxidant enzyme activity*

Leaf samples from each treatment were collected, wrapped in aluminum foil, and stored at -80°C for subsequent analysis of superoxide dismutase (SOD) and peroxidase (POD) activities.

For the determination of SOD activity using the nitroblue tetrazolium (NBT) method, accurately weigh 0.2 g of soybean leaves, stem tips, and root tips, and homogenize them under cold conditions. Transfer the homogenate to a centrifuge tube and centrifuge at 8000 r/min for 15 minutes at 4°C. Use 0.1 ml of the supernatant for the colorimetric reaction. Measure the absorbance at 560 nm, with three replicates, to calculate SOD activity. For the determination of POD activity using the guaiacol method, the extraction method for POD enzyme solution is the same as for SOD. After extraction, add 40 μl of enzyme solution to 3 ml of POD reaction solution. Measure the change in absorbance at 470 nm every 30 seconds, record four readings, with three replicates, to calculate POD activity.

### ***Data analysis and mapping software***

Data processing and analysis were performed with Excel 2013 and SPSS 20.0. Specifically, Analysis of Variance (ANOVA) was used to assess the impact of different treatments on the measured variables and to determine significant differences between treatments. Post hoc tests, including Tukey's Honest Significant Difference (HSD) test, were conducted to identify significant differences between treatment groups. The software used for mapping was Excel 2013.

## Results and analysis

### ***Effects of different degrees of water control on dry matter accumulation in soybean***

As can be seen from *Table 3*, dry matter accumulation of soybean continued to increase with the growth process, while drought stress reduced dry matter accumulation of soybean. As soil water potential decreased, dry matter accumulation in leaves and roots first increased and then decreased during the V1 period. The dry leaf weight of -15kp (T3) was 9.40% lower than which of CK. The dry weight of -5 KP in root under mild drought stress (T1) was significantly higher than that of CK and increased by 22.67%. Compared with CK, the dry weight of T3 treatment was reduced by 26.67%, and the difference reached a significant level (P<5%). Compared with the control, dry matter in the stems for T2 and T3 treatments decreased significantly by 11.86% and 20.34%, respectively. Dry matter in the stem was not significantly different compared to the control. Dry matter accumulation in leaves and roots had no significant difference compared with CK except T3 treatment. It can be seen that the accumulation of root dry matter is the most important factor in V1 period. In R1 stage, with the decrease of soil water potential 5, the dry matter of all parts of soybean plant decreased, among which the dry matter of leaves had the most obvious change. Compared with the control, the dry matter of T2 and T3 treatments decreased by 7.85% and 24.44%, respectively, and both reached significant differences (P<5%). There was no significant difference of dry matter in stems of all treatments. The root dry matter at -15kp (T3) was significantly reduced by 36.94% compared with CK. After rehydration, dry matter of leaves treated with -10kp (T2) recovered to no significant difference from CK. In R5 stage, soil water potential decreased and dry matter in all parts of soybean plant decreased, the most obvious


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changes were leaf and pod weight. The dry matter accumulation of leaves and pods under T2 and T3 treatments was significantly lower than which of the control, decreased by 22.36%, 25.45% and 19.19%, 40.71%, respectively. The change of stem dry matter accumulation was not obvious. The root dry matter of T3 treatment was significantly reduced by 29.22% compared with CK treatment. After rehydration, the dry matter accumulation in each part of the plant was not significantly recovered.

***Table 3.*** *Effects of different degrees of water control on dry matter accumulation in soybean (ANOVA, N=3)*

| | Treatments | Stage | | | | | |
|---|---|---|---|---|---|---|---|
| | | V1 | | R1 | | R5 | |
| | | Water controlling | Rehydration | Water controlling | Rehydration | Water controlling | Rehydration |
| Leaf weight/g | CK | 1.49±0.05ab | 3.06±0.22a | 4.46±0.19a | 5.20±0.29a | 5.50±0.34a | 5.13±0.39a |
| | T1 | 1.71±0.09a | 3.00±0.06ab | 4.45±0.20ab | 5.08±0.11a | 5.20±0.09a | 4.47±0.28ab |
| | T2 | 1.49±0.08ab | 2.89±0.22ab | 4.11±0.51b | 4.65±0.22ab | 4.27±0.20b | 3.47±0.28bc |
| | T3 | 1.35±0.15b | 2.48±0.10b | 3.37±0.14b | 4.06±0.13b | 4.10±0.40b | 3.10±0.43c |
| Stem weight/g | CK | 0.59±0.01ab | 1.02±0.11a | 2.95±0.26a | 3.73±0.03a | 4.90±0.10a | 4.81±0.34a |
| | T1 | 0.61±0.04a | 1.08±0.07a | 2.79±0.19a | 3.57±0.19ab | 4.70±0.31a | 4.61±0.56a |
| | T2 | 0.52±0.02bc | 0.96±0.05a | 2.69±0.33a | 3.20±0.15b | 4.30±0.39a | 4.18±0.27ab |
| | T3 | 0.47±0.02c | 0.94±0.03a | 2.23±0.07a | 2.79±0.04c | 3.99±0.29a | 3.75±0.35b |
| Root weight/g | CK | 0.75±0.01b | 1.13±0.01a | 3.33±0.47a | 3.92±0.17a | 5.27±0.61a | 5.17±0.20a |
| | T1 | 0.92±0.06a | 1.21±0.07a | 3.10±0.19a | 3.67±0.61ab | 5.17±0.30a | 4.84±0.16a |
| | T2 | 0.78±0.03ab | 1.18±0.09a | 2.91±0.11ab | 3.38±0.01ab | 4.58±0.47ab | 4.42±0.24ab |
| | T3 | 0.55±0.06c | 0.88±0.04b | 2.10±0.03b | 2.65±0.12b | 3.73±0.12b | 3.56±0.50b |
| Pod weight/g | CK | - | - | - | - | 11.57±0.53a | 14.78±0.93a |
| | T1 | - | - | - | - | 9.98±0.49ab | 13.96±0.87a |
| | T2 | - | - | - | - | 9.35±0.60b | 10.80±1.21b |
| | T3 | - | - | - | - | 6.86±0.73c | 8.07±0.68b |

Note: Table data represent longitudinal comparison of different treatments for the same organ. Different letters represent a significant difference level of 5% between treatments.

### *Effects of different degrees of water control on soybean root vitality*

Water stress can make the root growth adaptive change, and drought can enhance the root growth ability in a certain range. *Figure 2* demonstrates that various degrees of water control at different stages differently affected root vitality. With the continuous advancement of growth process, root vitality increased firstly and then decreased. In the V1 stage, with the decrease of soil water potential, the root vitality of soybean increased firstly and then decreased, and the root activity reached the maximum under -10kp (T2) treatment. T1 and T2 treatments were significantly higher than the control, increasing by 12.44% and 22.96%. However, root vitality in the T3 treatment decreased due to excessive soil water deficit. After rehydration, the root activity of T3 treatment was significantly higher than which of CK. Therefore, drought at seedling stage was beneficial


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

for the improvement of root vitality. In R1 stage, soybean root vitality increased with the decrease of soil water potential. At this stage, soybean root was relatively large, and water stress was conducive to the improvement of root vitality. Compared with CK, each treatment in R1 stage significantly increased by 12.75%, 29.69% and 35.44%. In R5 stage, at this time, root growth capacity decreased, and root vitality continuously decreased with the decrease of soil water potential. Compared with CK, T3 treatment significantly decreased, and there was no significant difference between the recovery of root vitality and CK after normal water supply.

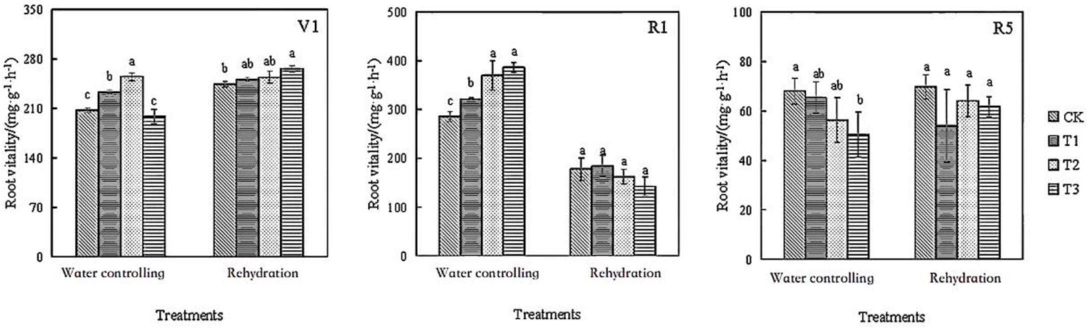


***Figure 2.*** *Effects of different degrees of water control on soybean root vitality. Note: different letters indicate significant difference between treatments at the level of 5%, the same as below. There were no significant differences between the data from the two years, and the trends were consistent*

### *Effects of different degrees of water control on photosynthesis of soybean plants at different periods*

#### *Effects of different degrees of water control on gas exchange parameters of soybean at V1 stage*

As shown in *Figure 3*, the net photosynthetic rate, transpiration rate and stomatal conductance of soybean leaves in the V1 stage increased by 7.9%, 16.4% and 1.0% respectively under mild drought-controlled water. As soil drought intensified, the photosynthetic parameters decreased. The net photosynthetic rate and stomatal conductance of T3 treatment decreased by 45.89% and 52.43% compared with CK, both of which reached a significant difference level ($P<0.05$). Compared with CK, the intercellular carbon dioxide concentration of -10kp (T2) treatment was significantly reduced by 12.89%, and the transpiration rate of T2 and T3 was significantly reduced by 23.91% and 51.75% compared with CK. In conclusion, the decrease of soil water potential in this experiment had the greatest effect on the leaf gas exchange parameters of soybean, including net photosynthetic rate, stomatal conductance and transpiration rate. After rehydration, there was an overall increase in the net photosynthetic rate. Specifically, the net photosynthetic rate in the T2 treatment was significantly higher than that of the control. The overall net photosynthetic rate was not significantly different from the control. In addition, after rehydration in T2 treatment, stomatal conductance and transpiration rate returned to the control level, with no significant difference compared with CK.


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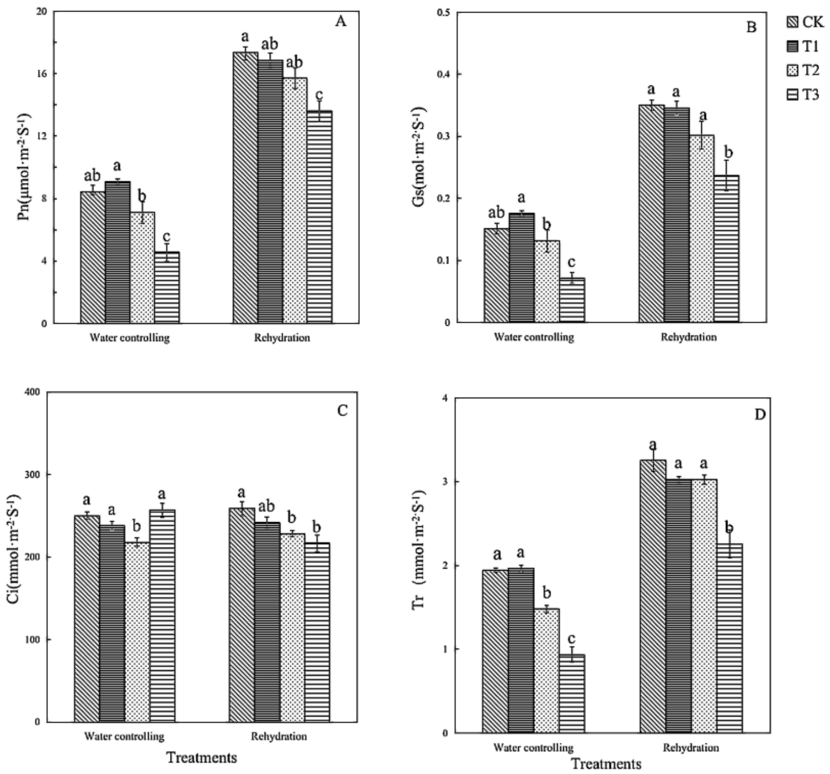


***Figure 3.*** *Effects of different degrees of water control on gas exchange parameters of soybean in stage V1. Note: different letters indicate significant difference between treatments at the level of 0.05, the same as below. There were no significant differences between the data from the two years, and the trends were consistent*

## *Effects of different degrees of water control on gas exchange parameters of soybean at R1 stage*

As can be seen from *Figure 4*, the decrease of soil water potential in R1 stage leads to the decrease of soybean leaf gas exchange parameters with varying degrees, among which Pn, Gs, Tr, under -10kp (T2) and -15kp (T3) treatment are reduced by 38.95%, 60.74%, 42.79%, 60.10%, 21.29%, 44.85%, respectively, compared with CK. In conclusion, the effects of drought stress on net photosynthetic rate, stomatal conductance and transpiration rate of soybean leaves at V1 stage were greater than those on intercellular carbon dioxide concentration. This may be attributed to the decrease of soil water potential, chlorophyll content and relative moisture content of soybean leaves, which inhibited the photosynthesis of soybean leave, subsequently leading to the decrease of stomatal conductance and net photosynthetic rate. The respiration of leaves also produces carbon dioxide, so the decrease of intercellular carbon dioxide concentration is smaller than which of net photosynthetic rate and stomatal conductance. After rehydration, the net photosynthetic rate, transpiration rate, stomatal conductance and intercellular carbon dioxide concentration of soybean leaves were increased compared with those before rehydration, but did not reach significant level.


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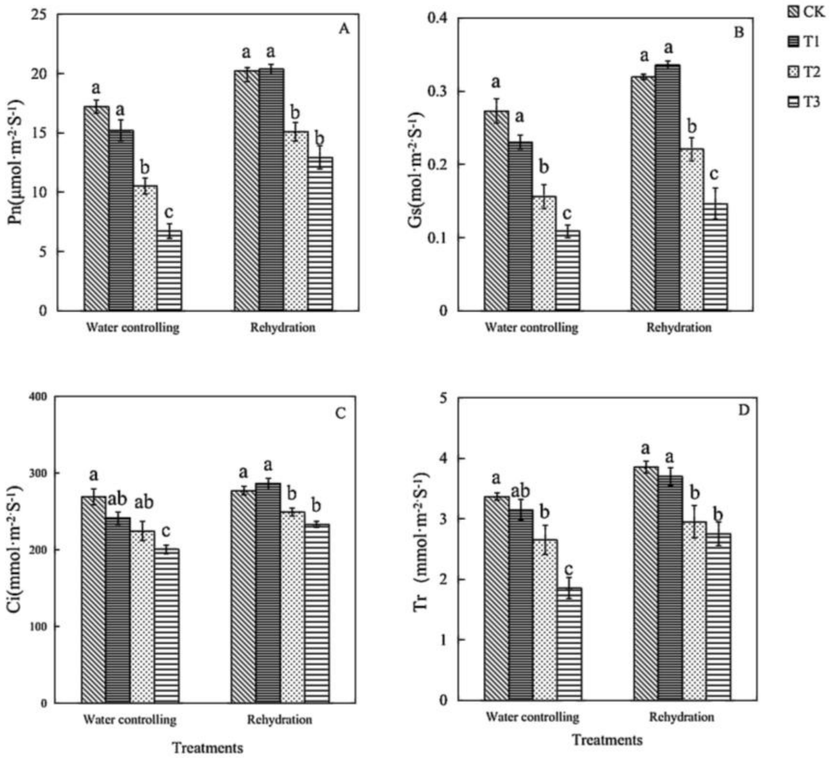


***Figure 4.*** *Effects of different degrees of water control on gas exchange parameters of soybean in R1 stage. Note: different letters indicate significant difference between treatments at the level of 0.05, the same as below. There were no significant differences between the data from the two years, and the trends were consistent*

*Effects of different degrees of water control on gas exchange parameters of soybean R5 stage*

As can be seen from *Figure 5*, the decrease of soil water potential leads to the decrease of gas exchange parameters of soybean leaves in R5 stage with varying degrees. Pn and Gs under -10kp (T2) and -15kp (T3) treatment are reduced by 38.73%, 69.46%, 36.10% and 53.83% compared with CK, respectively, reaching significant differences($P<0.05$). Compared with CK, the transpiration rate of T1, T2 and T3 decreased by 29.54%, 54.97% and 63.53%, respectively. With the decrease of water potential, the intercellular carbon dioxide firstly decreased and then increased. Compared with CK, T1 treatment significantly decreased by 17.44%, and T3 treatment significantly increased by 13.64%. The increased of Ci may be related to non-stomatal limitation. This indicated that the photosynthetic system and leaf tissue had been damaged by long-term water stress, and the carbon dioxide in the air entered into the leaf directly through the gaps in the leaves, which inhibited the absorption of light energy and reduced the normal operation of photosynthesis and carbon assimilation process. After rehydration, the net photosynthetic rate, transpiration rate and stomatal conductance of soybean leaves were increased compared with those before rehydration. Among them, the stomatal conductance of T2 treatment recovered to no significant difference compared with CK.


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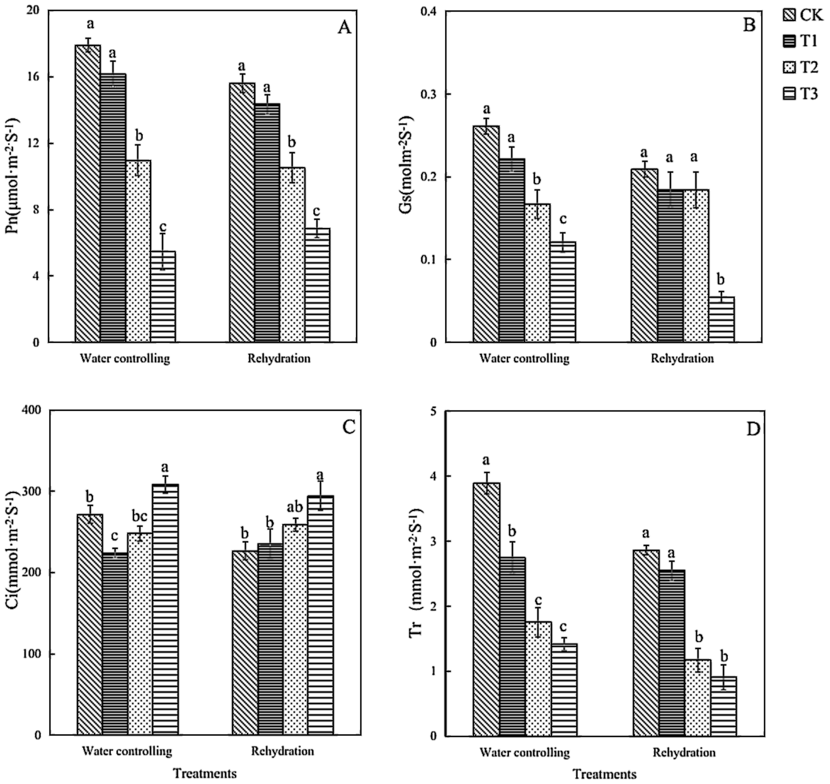


***Figure 5.*** *Effects of different degrees of water control on gas exchange parameters of soybean in R5 stage. Note: different letters indicate significant difference between treatments at the level of 0.05, the same as below. There were no significant differences between the data from the two years, and the trends were consistent*

## *Effects of different levels of water control on SOD activity in soybean leaves at V1, R1, and R5 stages*

As shown in *Figure 6*, water stress led to an increase in superoxide dismutase (SOD) activity in soybean, indicating that the plant's defense system was activated under water stress. With increasing levels of water stress, SOD activity in soybean leaves at the V1 stage showed a continuous upward trend. Compared to the control (CK), SOD activity in the -10 kPa (T2) and -15 kPa (T3) treatments increased significantly by 12.15% and 15.89%, respectively. After rehydration, SOD activity in all treatments returned to levels not significantly different from CK, suggesting that SOD activity in soybean leaves essentially returned to normal levels after rehydration.

Similarly, at the R1 stage, SOD activity in soybean leaves showed a continuous upward trend with increasing water stress. Compared to CK, SOD activity in the T3 treatment increased significantly by 21.66%.

At the R5 stage, under soil water stress conditions, SOD activity in soybean leaves first increased and then decreased, reaching its peak under the T2 treatment, which was 35.08% higher than CK. The increase in SOD activity under the T2 treatment was significant compared to CK. After rehydration, there were no significant differences in SOD activity among the treatments.


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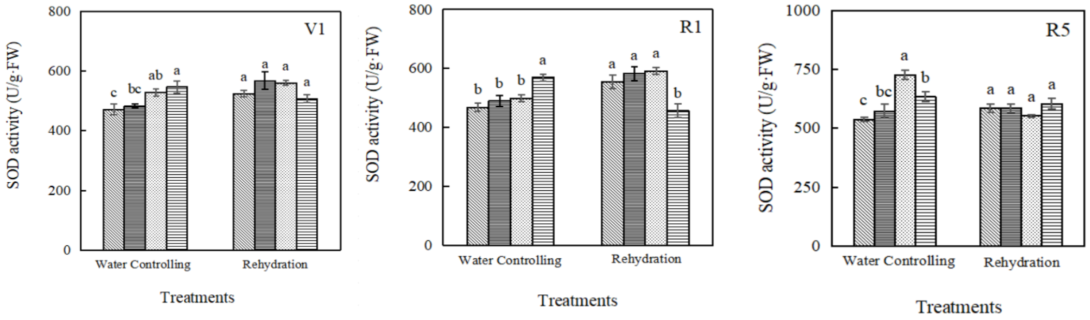


***Figure 6.*** *Effects of Different Levels of Water Control on SOD Activity in Soybean Leaves at V1, R1, and R5 Stages*

### *Effects of different levels of water control on POD activity in soybean leaves at V1, R1, and R5 stages*

As shown in *Figure 7*, under soil water stress, the peroxidase (POD) activity in soybean leaves at the V1 stage initially increased and then decreased. Compared to the control (CK), POD activity in the -5 kPa (T1) and -10 kPa (T2) treatments increased significantly by 41.67% and 57.14%, respectively, reaching significant differences. After rehydration, POD activity in the T3 treatment leaves was significantly higher than CK by 28.05%.

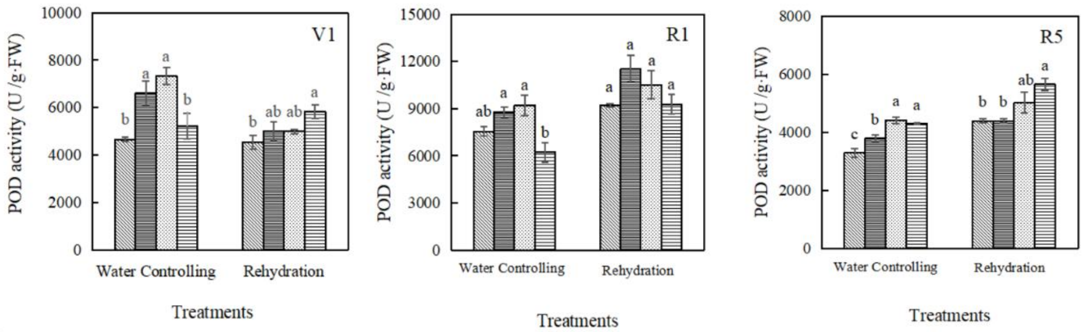


***Figure 7.*** *Effects of Different Levels of Water Control on POD Activity in Soybean Leaves at V1, R1, and R5 Stages*

At the R1 stage of soybean leaves, POD activity exhibited an initial increase followed by a decrease as soil water potential decreased, peaking at -10 kPa (T2 treatment), with an increase of 22.06% compared to CK. After rehydration, POD activity in the leaves increased overall, with no significant differences among the treatments compared to CK.

At the R5 stage, POD activity in soybean leaves showed an initial increase followed by a decrease as water potential decreased. Specifically, compared to the control, POD activity in the -10 kPa (T2) treatment increased significantly by 34.12%, reaching a significant difference.

### *Effects of different degrees of water control on soybean yield*

As can be seen from *Table 4*, different degrees of water stress in different periods have certain influences on the yield component factors, and the yield and yield component


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

factors in 2019 are analyzed: When water was controlled in stage V1, grain weight per plant under -5kp (T1) treatment was significantly increased by 8.97% compared with CK, grain number per plant and 100 grain weight were significantly higher than CK, increasing by 7.50% and 4.75%, respectively. The grain weight per plant under -15kp (T3) treatment significantly decreased by 5.86% compared with CK, pod number per plant, 100 grain weight per plant significantly decreased compared with CK.

***Table 4.*** *Effects of different degrees of water control on soybean yield and its constituent factors (ANOVA, N=3)*

| Stage | | Treatment | Pods per plant(g) | Seeds per pod | 100-grain weight (g) | Grain weight per plant(g) |
|---|---|---|---|---|---|---|
| 2019 | V1 | CK | 23.83±1.31a | 44.42±0.87b | 19.17±0.04b | 8.70±0.16b |
| | | T1 | 23.17±1.11a | 47.75±1.72a | 20.08±0.25a | 9.48±0.06a |
| | | T2 | 22.42±1.23ab | 44.97±2.2ab | 19.02±0.23b | 8.55±0.12b |
| | | T3 | 20.58±1.35b | 44.75±1.48ab | 17.85±0.13c | 8.19±0.07c |
| | R1 | CK | 23.83±1.31a | 44.42±0.87a | 19.17±0.04b | 8.70±0.16a |
| | | T1 | 23.50±1.71a | 45.25±1.07a | 19.99±0.11a | 8.95±0.21a |
| | | T2 | 19.33±1.07b | 41.25±1.23b | 18.63±0.12c | 7.68±0.31b |
| | | T3 | 18.83±0.91b | 39.42±1.44b | 18.60±0.25c | 7.33±0.27b |
| | R5 | CK | 23.83±1.31a | 44.42±0.87a | 19.17±0.04a | 8.70±0.16a |
| | | T1 | 21.33±0.82b | 45.46±2.80a | 18.66±0.18b | 8.43±0.30ab |
| | | T2 | 20.67±1.37bc | 42.27±1.93ab | 18.35±0.26b | 8.02±0.37b |
| | | T3 | 18.25±1.07c | 39.76±2.73b | 17.23±0.07c | 7.22±0.50c |

When water was controlled in R1 stage, grain weight per plant under -5kp (T1) treatment increased by 2.87% compared with CK. Compared with CK, the 100 grain weight was significantly increased by 4.28%. Grain weight per plant under -10Kp (T2) and -15Kp (T3) treatment decreased significantly compared with CK. Water control in stage R5, the grain weight per plant decreased in all places, and T2 and T3 treatment decreased significantly, by 7.82% and 17.01% respectively compared with CK.

It can be seen from *Table 5* that the analysis of yield and yield component factors in 2020 shows that, compared with CK, pod number per plant and grain number per plant of water-controlled soybean in V1 stage are significantly decreased under -10kp (T2) and -15kp (T3) treatment, decreasing by 7.3%, 10.2%, 15.8% and 14.3%, respectively. The 100 grain weight increased first and then decreased with the increase of water stress. Compared with CK, -5kp and -10kp treatment increased by 5.74% and 2.74%, respectively. Grain weight per plant under -10kp and -15kp treatment was significantly lower than the control, while grain weight per plant under -5kp treatment was increased by 5.0% compared with CK. It can be seen that proper drought-resistance exercise at seedling stage and normal water supply at later stage are helpful to increase yield. In R1 stage, the yield component was reduced. Compared with CK, pod number per plant, grain number per plant and grain weight per plant under -10kp and -15kp treatment were reduced by 13.66%, 15.26%, 11.5%, 20.28%, 20.68% and 16.97%, respectively. The seed number per plant, seed weight per plant and 100 seed weight in T1 treatment were decreased but not significant compared with CK, and pod number per plant was increased by 0.59% compared with CK. The R5 stage is crucial for grain development, and water


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

shortage during this stage leads to a reduction in yield. Compared with CK, each treatment reduces pod number per plant, grain number per plant and grain weight per plant by 5.4%, 3.4%, 3.4%, 2.9%, 24.9%, 8.2%, 6.8%, 26.9%, respectively. The pod number per plant and grain number per plant did not decrease significantly compared with CK. Compared with CK, the main reason for the decrease of yield of -10kp and -15kp treatment caused by water control in R5 stage was the significant decrease of 100 grain weight.

***Table 5.*** *Effects of different degrees of water control on soybean yield and its constituent factors (ANOVA, N=3)*

| Stage | | Treatment | Pods per plant(g) | Seeds per pod | 100-grain weight (g) | Grain weight per plant(g) |
|---|---|---|---|---|---|---|
| 2020 | V1 | CK | 20.38±0.64a | 49.80±1.42a | 19.35±0.03ab | 9.47±0.17a |
| | | T1 | 20.88±1.08a | 49.50±1.73a | 20.46±0.11a | 9.94±0.34a |
| | | T2 | 18.90±0.78b | 44.70±0.72b | 19.88±0.38a | 8.45±0.30b |
| | | T3 | 17.14±0.26c | 42.71±0.87b | 19.16±0.12b | 7.65±0.23c |
| | R1 | CK | 20.38±0.64a | 49.80±1.42a | 19.35±0.03a | 9.47±0.17a |
| | | T1 | 20.50±0.29a | 46.0±0.71ab | 19.23±0.06ab | 9.11±0.11ab |
| | | T2 | 17.60±0.51b | 42.2±0.97bc | 18.67±0.25ab | 8.38±0.55bc |
| | | T3 | 16.25±0.63b | 39.50±0.65c | 18.34±0.47b | 7.86±0.50c |
| | R5 | CK | 20.38±0.64a | 49.80±1.42a | 19.35±0.03a | 9.47±0.17a |
| | | T1 | 19.27±0.73a | 48.10±1.56a | 18.83±0.26ab | 8.40±0.46b |
| | | T2 | 19.69±0.33a | 48.38±1.14a | 17.25±0.08b | 7.11±0.15c |
| | | T3 | 18.71±1.18ab | 46.43±2.37ab | 15.38±0.59c | 6.92±0.25c |

Through the analysis of two years' yield, it was found that mild drought of -5kp (T1) had the best effect on the increase of yield, and the water control period was the best in V1 stage, followed by R1 stage, and the yield decreased obviously in R5 stage under drought stress.

## Discussion

### *Influence of different degrees of water control on soybean growth*

Drought stress primarily affects the plant root system, triggering the transmission of ABA (abscisic acid) signals, thereby impacting cellular enzyme activity and carbon metabolism assimilation transport (Zhang et al., 2007). This leads to adaptive morphological changes in the root system to reduce plant biomass in response to the drought environment. Simultaneously, as the core area for dry matter production, soybean leaves under drought conditions show a significant reduction in dry matter accumulation, which can rapidly recover after normal water supply (Zhang et al., 2018). Drought stress adversely affects soybeans throughout their entire developmental period. Soybean seedlings, which have high water requirements, are impacted in traits such as total root length during the seedling stage, while drought during the flowering stage affects characteristics like stem thickness. Drought throughout the entire growing period comprehensively impacts traits such as plant height (Fan, 2023). During the seedling growth stage, plants treated with 50-65% water content exhibited the greatest growth in height (4.50 cm), while those with 90-100% field capacity showed the least growth in


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

height (1.50 cm), due to excessive soil moisture. In the pod-bearing stage, plant height growth was slow, with the maximum growth (73.6 cm) observed under the 75-85% water content treatment. Therefore, during the seedling stage, lower levels of water are more beneficial for plant growth (Hai, 2019).

Moreover, this study found significant differences in the impact of drought on different growth stages of soybean. During the flowering and pod-filling stages, drought significantly inhibited dry matter accumulation, with the effect being most prominent during the flowering stage (Cai, 2004). During the seedling stage, however, mild drought stress actually promotes dry matter accumulation in both leaves and roots. This may be related to drought-induced root system development and enhanced nitrogen, phosphorus, and potassium absorption capabilities. It is noteworthy that soybean seedlings require a longer recovery time after severe drought, affecting the growth rate and dry matter accumulation. In contrast, plants during the flowering stage have stronger resistance to adversity and recover quickly after drought relief. The pod-filling stage is a critical period for dry matter transport, during which drought resistance is the weakest. Even with re-watering, the compensatory effects under severe stress are not significant. Overall, the impact of drought stress on plant growth is complex and clearly stage-dependent, with different growth stages exhibiting varying responses and recovery capabilities to drought. This provides important references for future agricultural management.

### *Effects of different degrees of water control on soybean photosynthesis*

Photosynthesis is a key energy metabolism system for crop growth and directly affects yield. Stomata serve as the primary conduits and regulators for the exchange of water vapor between plants and their external environment. By modulating water loss and $CO_2$ uptake in plants, stomata play a crucial role in plant adaptation to environmental changes (Liu et al., 2022). The opening and closing of stomata in leaves are regaled by RWC and tend to close with decreasing RWC resulting in lower Gs under drought (Fatema et al., 2023). During the flowering and pod-filling stages of soybean, the photosynthetic rate (Pn) tends to decrease with the intensification and prolongation of drought stress. Under drought stress during the flowering stage, the decline in soybean Pn is predominantly governed by stomatal factors, whereas in the pod-filling stage, the primary factor for the reduction shifts from stomatal to non-stomatal elements (Fatema et al., 2023). In the course of summer soybean development, Shang Kai's research demonstrates that both the net photosynthetic rate and stomatal conductance exhibit an initial increase followed by a decrease, while the transpiration rate progressively declines. With escalating water deficit, the concentration of intercellular $CO_2$ in the leaves also increases. This trend persists until the approach of the flowering stage in summer soybean, at which point the pattern reverses compared to earlier stages of growth (Shang, 2021).

This study found that under drought stress, plants reduce water loss by limiting stomatal aperture, decreasing stomatal conductance (Gs) and transpiration rate (Tr), leading to stomatal closure and reduced CO2 absorption, thereby limiting photosynthesis. Prolonged drought stress increases the intercellular CO2 concentration (Ci), suggesting damage to the photosynthesis system, while chlorophyll content decreases, weakening light absorption and photosynthetic capacity (Xu et al., 2004). In seedling-stage drought stress, mild stress paradoxically enhances photosynthesis (Pn), Tr, and Gs, possibly due to promoting root dry matter accumulation and transport of photosynthetic products to the roots. Moderate and severe drought stress, however, reduce photosynthetic capacity. After re-watering, Pn, Tr, and Gs under mildand moderate stress show no significant


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

difference compared to the control group (CK). During the flowering stage, drought stress of any degree reduces Pn, Gs, Ci, and Tr. However, after re-watering, these indicators are higher than CK under mild stress, possibly because moderate stress improves nutrient absorption and transport efficiency, maintaining higher photosynthetic capacity. Pod-filling stage findings show that soybean leaf photosynthetic capacity cannot recover after re-watering, likely due to severe drought-induced damage to absorption and photosynthesis, compounded by accelerated substance turnover, preventing restoration of photosynthetic capacity. In summary, the impact of drought stress on photosynthesis varies with stress degree and crop growth stage; appropriate drought treatment can enhance photosynthetic efficiency, but excessive stress causes irreversible damage.

### *Effects of different levels of water control on the antioxidant system in soybean*

Our study indicates that mild drought stress at different growth stages of soybean enhances the activity of antioxidant enzymes. During the seedling stage, severe drought stress results in the highest increase in antioxidant enzyme activity. At the flowering stage, mild drought stress significantly enhances antioxidant enzyme activity. During the pod-filling stage, antioxidant enzyme activity shows an increasing trend under mild and moderate drought stress, but a decreasing trend under severe drought stress. This decline is associated with accelerated plant maturation, increased substance transport, and accelerated senescence due to intensified drought stress during the pod-filling stage, leading to plant death and reduced antioxidant enzyme synthesis. The leaves, mainly due to decreased light utilization efficiency, experience delayed reduction of excess oxygen ions, generating reactive oxygen species (ROS).

When drought increases reactive oxygen species (ROS) in plant tissues, the plant's protective enzyme system is activated. Enzymes such as superoxide dismutase (SOD) show increased activity, clearing the accumulated ROS to maintain ROS levels within a normal range, thereby protecting the plant from damage. Under drought stress, the activity of protective enzymes in soybean significantly increases. However, the activity of antioxidant enzymes does not continuously rise; when drought stress exceeds a certain threshold, the activities of SOD and peroxidase (POD) show a declining trend.

### *Effects of different degrees of water control on soybean yield and quality*

Drought is one of the key limiting factors affecting soybean yield, reducing production through the regulation of plant physiological and biochemical responses. Under drought conditions, soybean roots perceive stress and produce chemical signals to regulate plant physiology, mitigating the damage caused by drought. When drought severity exceeds the plant's protective mechanisms, growth is significantly affected, leading to a substantial decrease in yield. Notably, drought impedes subterranean growth, reduces leaf area and chlorophyll content, and decreases photosynthetic efficiency (Li, 2019). Studies indicate that the pod stage is particularly sensitive to drought, as it is a critical period for soybean growth and development. During this stage, drought increases pod abortion rates, severely impacting yield. Other research also suggests that soybean is most sensitive to drought during the pod-forming stage, as drought lowers reproductive organ water potential, inhibiting pod growth (Ge, 2013). Yan et al. (2013) results show that the more severe the drought, the greater the yield reduction, and the impact of drought varies across different growth stages. In Ogunkanmi et al. (2022), it is demonstrated that under conditions of soil moisture deficit, the decline in pod and seed yields is attributed to the lack of adequate water necessary for normal growth and development, which may


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_215234

adversely affect photosynthetic processes. During periods of water stress, plants often close their stomata, resulting in decreased carbon dioxide uptake and subsequent decline in biomass accumulation. The more pronounced reduction in yields is likely due to the occurrence of water stress during the reproductive phase, a stage highly sensitive to such stress. This leads to diminished grain size and ultimately reduces grain yield (Ogunkanmi et al., 2021). Wang et al. (2020) discovered that as drought stress intensity and duration increased, the number of pods and grains per plant decreased, while the number of empty grains increased, reducing yield, with the impact of drought stress on soybean yield being greater during the pod-filling stage than the flowering stage. The primary reason is that drought stress affects the material distribution ratio among soybean organs, thus impacting reproductive growth and development (Wang et al., 2020), leading to a decrease in the number of pods and grains per plant and an increase in empty grains, ultimately reducing yield.

Our study shows that mild drought stress can increase yield in the seedling stage but decrease it during the pod-filling stage, related to the reduction in pod number, grain number, and hundred-grain weight. Moderate and severe droughts generally reduce yield. The loss in yield is the result of a combination of factors, including growth and development, photosynthesis, material transport, and assimilation synthesis. The increase in yield under mild drought stress is mainly due to enhanced photosynthetic capacity and dry matter accumulation inroots and leaves, less associated with antioxidant enzyme activity, and more with nitrogen content. As soybean is an important oil crop, its impact on protein and fat content also warrants attention. Previous studies have shown that drought stress increases protein content while decreasing fat content in soybeans (Cui et al., 2013). Our analysis of different periods and degrees of controlled water treatment found that the impact of drought stress on seed protein is less than that on fat content. As soil water potential decreases, seed protein content increases while fat content decreases, associated with enhanced nitrogen transport and organic acid transport and degradation.

### *Future prospect*

The research on the impact of various degrees of water deficit on soybean growth and yield holds significant importance for enhancing the efficiency of agricultural water resource use, optimizing irrigation management and agricultural planning, and in formulating agricultural policies. The research outcomes can guide farmers and agricultural managers in adjusting their irrigation strategies, thereby reducing water resource wastage and providing decision support for cultivation in areas with limited water resources. Future studies should pay more attention to long-term effects, experiments under diverse environmental conditions, development of drought-resistant varieties, and comprehensive management strategies. Extensive-period experiments and multifaceted strategy research will aid in profoundly understanding the impact of water deficit on soybean growth, developing effective management strategies, and optimizing agricultural production, particularly in situations with limited water resources.

## Conclusion

Mild drought (-10kp) during the V1 stage promotes root vitality, increasing dry matter accumulation. Drought stress in the R5 stage significantly inhibits the dry matter accumulation in leaves, with weak recovery capacity after rewatering. Mild drought stress in the seedling stage can enhance photosynthesis, but drought during the flowering stage


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DOI: http://dx.doi.org/10.15666/aeer/2301_215234

reduces photosynthetic parameters, and the photosynthetic capacity is difficult to fully recover after rewatering. Drought stress causes excessive accumulation of reactive oxygen species (ROS) in soybeans, with the amount of ROS increasing as the stress intensifies. At different growth stages, mild drought stress has been shown to enhance the activity of antioxidant enzymes. During the seedling stage, severe drought stress results in the highest increase in antioxidant enzyme activity, which alleviates oxidative damage. During the pod-filling stage, antioxidant enzyme activity shows an increasing trend under mild and moderate drought stress but a decreasing trend under severe drought stress. Yield analysis shows that -5kp drought stress (especially during the V1 stage) can increase yield while causing an increase in seed protein content and a decrease in fat content. Therefore, scientific water management through short-term drought stress can achieve water-saving and yield-increasing effects.

**Acknowledgments.** This work was supported by the National Key R&D Projects (2022YFD1000105), Natural Science Foundation of Heilongjiang Province (LH2022C063) Project funded by China Postdoctoral Science Foundation (2022M720695).

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