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Effects of drought stress on flowering soybean physiology under different soil conditions

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Abstract: Soybean is highly sensitive to drought stress during its flowering period. Heinong84 (HN84) and Hefeng46 (HF46) were planted in clay loam, silty loam, and sandy clay. We studied the effects of drought stress on the content of membrane lipid peroxides in flowering soybean leaves, the activity of antioxidant enzymes, and the activity of key enzymes of nitrogen metabolism under different soil conditions. Our results showed that soybean had clear physiological responses to drought stress. With increasing drought stress, the malondialdehyde, glutathione reductase, and glutathione peroxidase levels in soybean leaves increased continuously. Superoxide dismutase, peroxidase, glutamine synthase, and glutamate synthase levels increased with drought stress, reaching a maximum under moderate drought stress and then decreased; nitrate reductase activity decreased continuously. Under the condition of sufficient water, the performance of soybean in the three soils is almost the same, but there are differences under drought stress; particularly, soybean grown in clay loam shows the strongest drought resistance. In summary, the physiological state of soybean is easily affected by drought stress, which varies greatly among different cultivars and in different soil types.

Keywords: water deficit; crop; enzymatic activity; physiological characteristics

Droughts often occur in China. In 2010, local droughts occurred in major agricultural-producing areas in many areas of China, which had a great impact on food and economic losses (Huang et al. 2012). In addition, global warming and the increase of greenhouse gases have intensified the stress of drought on crops (Xu and Zhou 2010). Soybean (*Glycine max* L.) is an important food crop and cash crop with high nutritional value (Zha et al. 2018). However, as a crop with higher water requirements, soybean is especially sensitive to water deficit. The organ balance, economic coefficient, and soybean yield will likely be affected by drought stress. The predicted effect varies between mild and severe stress (Bai et al. 2009). Drought stress affects soybean seed germination and seedling growth leading to smaller grain size, a decrease in 100-grain weight, and yield reduction (Mo et al. 2014, Guo et al. 2015). In addition,

drought stress can also lead to changes in crop nutrient content; for example, the oleic acid content of soybean grain decreased slightly as well as the content of linoleic acid and linolenic acid increased when the flowering period was under drought stress (Cui et al. 2012).

Drought stress leads to the increase of membrane lipid peroxidation, and malondialdehyde (MDA), as a natural product of membrane lipid peroxidation, is an important indicator to detect whether damage to the cell membrane has occurred (Jiang et al. 2001). Crops with higher drought resistance usually have lower MDA content under drought stress (Ma et al. 2015). Malondialdehyde content increases under high temperature, drought, low temperature, and salt stress (Guo et al. 2018, Rui et al. 2018). Reactive oxygen species (ROS) are molecules or ions with high chemistry activity. Under

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drought conditions, mitochondria or chloroplasts produce a large number of ROS, which transform into highly toxic hydroxyl radicals and destroy cell membranes and proteins and nucleic acids if they are not excreted in time (Simova-Stoilova et al. 2009). Peroxidase (POD), superoxide dismutase (SOD), and glutathione peroxidase (GPX) are key antioxidant enzymes that protect crops against drought stress. They aid in the removal of reactive oxygen species produced by plants under stress and reduce or eliminate the damage caused by superoxide anions to cells, and protect crops from drought stress (Dong et al. 2015).

Studies have found that some nitrogen metabolic reductases of soybean also play a crucial role in soybean resistance to drought stress (Xu et al. 2016). Nitrate reductases can reduce NO_3^- absorbed by roots to NO_2^- , which is the first step in the process of nitrogen metabolism. They are also rate-limiting enzymes in the nitrogen metabolism cycle. Under drought conditions, a decrease in nitrate reductase (NR) activity has an absolute limiting effect on the nitrogen metabolism process of soybean (May et al. 2011). Glutamine synthase is central to nitrogen metabolism, and inorganic nitrogen must be catalysed into glutamine by glutamine synthase (GS) before it can be utilised by plants (Chen et al. 2010). Inorganic nitrogen sources are assimilated through the glutamate synthase (GS-GOGAT) pathway and eventually converted into amino acids and absorbed (Bernard and Habash 2009, Wang et al. 2017). The NR, GS, and GOGAT activity levels are key indicators of the nitrogen metabolism capacity of plants. The

activity of nitrogen metabolism enzymes can reflect the nitrogen metabolism capacity of plants (Chen et al. 2010). The activity of nitrogen metabolism enzymes can indicate the intensity of the drought stress suffered by the crop.

The soybean is greatly affected by its growth environment. Silty loam is a low-yield soil with high viscosity and acidity. Sandy clay and clay loam are mainly composed of clay and loam (Xiu et al. 2021). The total porosity of clay loam and sandy clay is about half of all the soil, and the water permeability is relatively low (Zhou et al. 2010). Therefore, it is necessary to investigate the effects of the type of soil on soybean growth. In this experiment, the growth of two different soybean cultivars (HN84 and HF46) on three different soils (clay loam and sandy clay and silty loam) was studied to explore the changes of soybean physiological indices under different degrees of drought stress. This study will provide an important theoretical basis for research into the suitability of different soil types for soybean cultivation and the selection of drought-resistant cultivars.

MATERIAL AND METHODS

Test materials. The experiment was carried out in the greenhouse of Northeast Agricultural University. The soybean used were HeFeng 46 (HF46) and HeiNong 84 (HN84), widely cultivated in Heilongjiang province. The test soil was silty loam, clay loam, and sandy clay.

Texture, pH, cation exchange capacity, organic carbon and other nutrients are shown in Table 1.

Table 1. Texture, pH, cation exchange capacity, organic carbon, total nitrogen, phosphorus, and potassium, available nitrogen, phosphorus, and potassium content in different soils

Soil texture (USA soil classification)	Clay loam	Sandy clay	Silty loam
clay (< 0.002 mm)	34.59	28.79	14.68
Particle size composition (%)			
silt (0.002–0.02 mm)	37.18	13.29	61.64
sand (0.02–2 mm)	28.23	57.92	23.68
pH	6.80	8.11	5.37
Cation exchange capacity (mmol/kg)	242.0	320.5	145.4
Organic carbon content (g/kg)	20.15	19.40	19.01
Total nitrogen (g/kg)	2.68	2.56	2.42
Total phosphorus (g/kg)	0.85	0.70	0.74
Total potassium (g/kg)	28.86	25.44	22.60
Available nitrogen (mg/kg)	254.73	221.77	201.71
Available phosphorus (mg/kg)	53.28	39.25	47.91
Available potassium (mg/kg)	247.12	225.49	180.32

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Test design. Full and uniform seeds were selected and placed in a plastic bucket with a hole at the bottom. The plastic bucket for the potted plant was 35 cm in height and 30 cm in diameter. Each bucket contained 14 kg of dried soil, free of weeds and rocks. In our experiment, there were two cultivars, three soil conditions and four water conditions, with a total of 24 treatments. Each treatment had three replicates, and each replicate was a mixture of four potted plants. A total of 288 potted plants were planted. Three soybean seedlings were placed in each pot. Before the flowering stage (R2 stage), the soil was kept under suitable moisture conditions in all buckets. After entering the R2 stage, the drought treatment began. The soil moisture meter ECH2O-TE/EC-TM (EM-50, Decagon, Washington, USA) and weighing method were combined to control and monitor the soil moisture content.

The experiment was divided into four treatments: control (CK); light drought (LD); moderate drought (MD), and severe drought (SD). The soil water content in CK is 70–80% of the field water capacity. The soil water content of the LD treatment was 50–60% of the field water capacity. The soil moisture content of the MD treatment was 40–50% of field water capacity. The soil water content of the SD treatment was 30–40% of the field capacity. Each treatment was sampled at the end of three days. The sampling time was from 8:00 a.m. to 9:00 a.m., and the samples were taken from the inverted second and third leaves (compound leaf) of soybean, with four experimental repetitions each. After procurement, the samples were rapidly frozen with liquid nitrogen, brought back to the laboratory, and stored in an ultra-low temperature refrigerator at $-80\text{ }^{\circ}\text{C}$ for later analysis.

Determination of physiological and biochemical indexes. The malondialdehyde content in the leaves was determined by the MDA kit (Grace Biotechnology, Suzhou, China). Weigh 0.1 g frozen sample, add 1 mL MDA extract ice bath grinding, $4\text{ }^{\circ}\text{C}$, 12 000 g centrifuge 10 min, take the supernatant, place on ice to be tested. Add 600 μL of the reaction solution and 400 μL of the extract to the centrifuge tube. $95\text{ }^{\circ}\text{C}$ water bath for 30 min. Cooling, $25\text{ }^{\circ}\text{C}$, 12 000 g centrifuge 10 min. The absorbance was read at 532 nm and 600 nm, respectively.

The activities of superoxide dismutase, peroxidase, glutathione reductase (GR), and glutathione peroxidase in leaves were determined by using the SOD, POD, GR and GPX kits (Grace Biotechnology, Suzhou, China). Weigh 0.1 g frozen sample, add 1 mL SOD,

POD, GR or GPX extract respectively when measuring SOD, POD, GR or GPX, then grind in the ice bath, centrifuge at $4\text{ }^{\circ}\text{C}$, 12 000 g for 10 min, take the supernatant and place it on the ice for testing. When measuring SOD activity, the crude enzyme solution and the specified reagent should be added to the centrifuge tube. After standing in the dark at $25\text{ }^{\circ}\text{C}$ for 30 min, the absorbance A_{SOD} was measured at 450 nm. When measuring POD activity, the absorbance value A_{POD} should be read at 470 nm immediately after adding crude enzyme solution and specific reaction solution to the centrifuge tube, and A'_{POD} should be read one minute later. Determination of GR enzyme activity should be added in the centrifuge tube after the crude enzyme solution and the specified reagent in a $25\text{ }^{\circ}\text{C}$ water bath for 10 min, and in the cuvette, in turn, to add the specified reaction solution, immediately mixed, at 412 nm wavelength of 30 s read the initial absorbance A_{GR} 10 min after measuring the absorbance A'_{GR} . Determination of GPX enzyme activity should be added in the centrifuge tube after the crude enzyme solution and the specified reagent after standing at $25\text{ }^{\circ}\text{C}$ for 5 min, and then add the specified reagent 12 000 g centrifuged for 10 min, the supernatant for testing. The absorbance A'_{GPX} was determined at 412 nm after the addition of the chromogenic agent according to the instruction. The absorbance of the blank tube was A_{GPX} .

The nitrate reductase, glutamine synthase, and glutamate synthase activities in leaves were determined by the NR, GS and GOGAT kits (Grace Biotechnology, Suzhou, China). Weighing 0.1 g frozen samples, 1 mL NR, GS or GOGAT extract was added to the determination of NR, GS and GOGAT, respectively, grinded in the ice bath, centrifuged at $4\text{ }^{\circ}\text{C}$, 12 000 g for 10 min, and the supernatant was placed on ice for testing. When NR activity was determined, the crude enzyme solution and the specified reaction solution was added according to the kit instructions, and the water bath was shaded at $30\text{ }^{\circ}\text{C}$ for 30 min. Then the mixed reaction solution was added, and the reaction was shaded at $30\text{ }^{\circ}\text{C}$ for 15 min. The absorbance A'_{NR} and A_{NR} were measured at 530 nm (A'_{NR} is the measuring tube, and A_{NR} is a reference tube). Determination of GS activity in accordance with the kit instructions to add enzyme solution and the specified three reaction solution, $37\text{ }^{\circ}\text{C}$ water bath for 30 min, and then add the fourth reaction solution, mixing, reaction 2 min, 8 000 g, $4\text{ }^{\circ}\text{C}$ centrifuged 10 min, at 540 nm absorbance A'_{GS} and A_{GS} (A'_{GS} is the measuring tube and A_{GS} is a reference

tube). When GOGAT activity was measured, the crude enzyme solution was added according to the kit instructions and mixed with the specified reaction solution. A_{GOGAT} was read after 1 min, and A'_{GOGAT} was read after 10 min. After the determination, the MDA content and enzyme activity was calculated according to the following formula:

$$\text{MDA content (nmol/g FW)} = [(A_{532} - A_{600})/(\epsilon_1 \times d) \times V_2 \times 109]/(W \times V_1/V) = 16.1 \times \Delta A/W$$

$$\begin{aligned} \text{inhibiting percentage} &= [(A \text{ blank tube 1} - A \text{ blank tube 2}) - (A \text{ sample tube} - A \text{ sample comparison tube}^*)]/(A \text{ blank 1} - A \text{ blank 2}) \times 100\%; \\ \text{SOD activity (U/g FW)} &= [\text{inhibiting percentage}/(1 - \text{inhibiting percentage}) \times V_2]/(W \times V_1/V) \times D = 12.5 \times \text{inhibiting percentage}/(1 - \text{inhibiting percentage})/W \times D \end{aligned}$$

$$\text{POD (U/min/g FW)} = (A'_{\text{POD}} - A_{\text{POD}})/(W \times V_1/V)/0.5/T = 50 \times \Delta A/W$$

$$\text{GR activity (nmol/min/g FW)} = [(A'_{\text{GR}} - A_{\text{GR}})/\epsilon_2/d/2 \times 109 \times V_2]/(W \times V_1/V)/T \times D = 36.8 \times \Delta A/W \times D$$

$$\text{GPX activity (nmol/min/g FW)} = [(A_{\text{GPX}} - A'_{\text{GPX}} - 0.0008)/5.3806 \times 103 \times V_2]/(W \times V_1/V)/T \times D = 464.6 \times (A_{\text{GPX}} - A'_{\text{GPX}} - 0.0008)/W \times D$$

$$\text{NR (nmol/h/g FW)} = [(A'_{\text{NR}} - A_{\text{NR}} + 0.0005)/0.8566 \times 103 \times V_1]/(W \times V_1/V)/T = 2\,335 \times (\Delta A + 0.0005)/W$$

$$\text{GS (U/g FW)} = (A'_{\text{GS}} - A_{\text{GS}})/(W \times V_1/V)/0.01/T = 16.7 \times \Delta A/W$$

$$\text{GOGAT (nmol/min/g FW)} = 2 \times [(A_{\text{GOGAT}} - A'_{\text{GOGAT}}) \times V_2/(\epsilon_3 \times d) \times 109]/(W \times V_1/V)/T = 3\,215.4 \times \Delta A/W/TV$$

Where: TV – total volume of sample extract, 1 mL; V_1 – sample volume added to the reaction system, mL; V_2 – total reaction liquid volume of sample extract and working solution, mL; d – light path of cuvette, 1 cm; ϵ_1 – MDA molar extinction coefficient, 155×103 L/mol/cm; W – sample quality, g; D – sample dilution multiple, undiluted is 1; T – reaction time, min; ϵ_2 – TNB molar extinction coefficient, 1.36×104 L/mol/cm; GSH molecular weight: 307.3; ϵ_3 – NADH molar extinction coefficient, 6.22×103 L/mol/cm.

Data analysis. All data were processed with Microsoft Office Excel 2010 (Redmond, USA) to make a histogram. The statistical analysis of the data was performed using IBM SPSS software (version 21.0: IBM Corporation, Armonk, USA) for Duncan's single-factor analysis of variance. OriginPro2021 (Origin Lab Corp., Northampton, USA) was used for Pearson correlation analysis.

RESULTS AND DISCUSSION

Effects of drought stress on malondialdehyde content in soybean. As shown in Figure 1, drought stress affects MDA content in soybean leaves. Levels of MDA content in soybean crop planted in three different soils increases with the decrease in soil moisture content, and MDA content reaches the maximum under SD conditions. Under SD conditions, MDA content in soybean is significantly higher than in the other experimental treatments. The MDA content of HN84 in SD increased by 129.8, 164.8, and 117.9% compared with that of the CK treatment in sandy clay, silty loam, and clay loam, respectively. The MDA content of HF46 in SD increased by 142.2, 191.0, and 134.6% compared with that of CK in sandy clay, silty loam, and clay loam, respectively. Under the SD treatment, the increase in MDA was greater in HF46 in different soils than in HN84. After drought stress was applied, the MDA content of soybean grown in clay loam increased the least, except for HF46 in MD condition; the MDA content of both kinds of soybean in clay loam was significantly lower than in silty loam.

Drought stress can cause a series of physiological changes in crops. Physiological analysis of plant drought stress responses is the primary way to reveal how stress affects crop growth and yield (Wang et al. 2022). Under drought stress, soybean membrane lipid peroxidation was strengthened (Dong et al. 2019), and MDA content increased (Gökmen and Ceyhan 2015, Katam et al. 2020, Farooq et al. 2021). In this experiment, different degrees of drought stress led to an increase in MDA content, and it reached the highest levels under severe drought, causing severe damage to the plant cell membrane. The MDA levels increased most slowly in clay loam, and soybean suffered the least drought-stress-related damage in the clay loam. This implies that soybean grown in clay loam is most resistant to drought-stress-related damage.

Effects of drought stress on antioxidant enzymes in soybean. The SOD activities of HN84 and HF46 grown in different soils and under different soil moisture conditions are shown in Figure 2A. Increasing drought stress results in soybean leaves showing a trend wherein SOD levels increase up to a point and then decrease. The SOD activity sequence is MD > SD > LD > CK, which is consistent in the three soil types. The SOD activity in MD was significantly higher than in other soil moisture states, while HF46 showed no significant difference between LD and SD.

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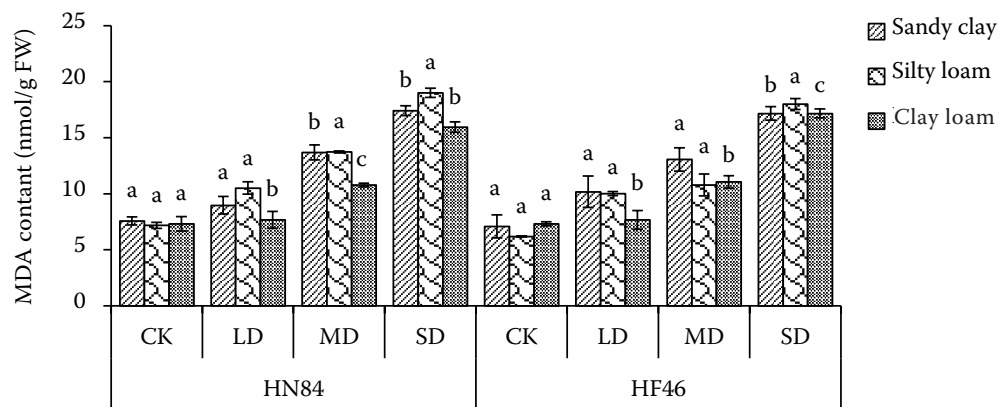


Figure 1. Malondialdehyde (MDA) content in soybean leaves planted in three soils under different water conditions. The level of significance is $P < 0.05$. CK – control; LD – light drought; MD – moderate drought; SD – severe drought. Error bars indicate standard error; FW – fresh weight

The cv. HN84 showed a significant difference between SOD activity in LD and SD states. The SOD activity of HF46 leaves under the MD state increased by 143.1, 141.7, and 158.4% compared to that seen in HF46 grown under CK conditions in sandy clay, silty loam, and clay loam, respectively. The SOD activity of HN84 in MD showed an increase of 140.5, 127.0, and 142.6% over that seen in HN84 grown under CK conditions in sandy clay, silty loam, and clay loam, respectively. Under the MD state, the increase in SOD activity of HF46 was greater than in HN84. Under CK, LD and SD states, the SOD activity of both soybean cultivars in clay loam was significantly higher than in the other two types of soil. Camaille et al. (2021) believed that under drought stress, wheat cells would produce a large number of antioxidant enzymes, such as SOD, to maintain the important functions of cells. Our experiment found that antioxidant enzyme levels in soybean leaves showed a significant increase under drought stress to resist the oxidative damage caused by drought stress. However, the activity of SOD did not increase continuously under drought stress. The study by Guzzo et al. (2020) showed that the activity of SOD in soybean leaves decreased immediately after reaching the peak under increasing drought. The possible reason is that the increase of SOD activity has been unable to resist severe drought stress, and too severe drought stress has damaged the antioxidant system of soybean, resulting in a significant decrease in SOD activity. Similar conclusions were also obtained in our experiments, and Anjum et al. (2017) also obtained similar conclusions when studying maize. But Hou et al. (2021) asserted that the activities of SOD de-

creased first and then increased with the increase of drought stress in *Carex*. This is different from the results of our experiments, which may be caused by the difference in drought stress degree, stress time, and species used in the test.

The POD activities of HN84 and HF46 grown in different soils and under different water conditions are shown in Figure 2B. Increasing drought stress results in soybean leaves showing a trend wherein POD activity first increases and then decreases. The POD activity in both soybean cultivars showed a pattern of MD > LD > SD > CK. The POD activity was significantly higher in MD than in the other water states, but there was no significant difference between POD activity at LD and SD states. The POD activity change was consistent in the three soil types. The POD activity of HF46 leaves in MD was 106.9, 115.5, and 88.0% greater than in HF46 grown in CK conditions in sandy clay, silty loam, and clay loam, respectively. The POD activity of HN84 in MD was 105.6, 110.5, and 63.8% higher than in HN84 grown under CK conditions in sandy clay, silty loam, and clay loam, respectively. In MD, the increase in POD activity in HF46 was higher than in HN84. Hou et al. (2021) experimented that the activity of POD in plant leaves was significantly increased under drought stress, and POD activity increased with drought stress. In the experiment of Zhang et al. (2021) and Zhou et al. (2022), the activity of POD was significantly increased in light or short-term drought stress conditions. However, too severe drought stress will seriously damage the antioxidant system of plants, resulting in a significant decrease in the activity of antioxidant enzymes such

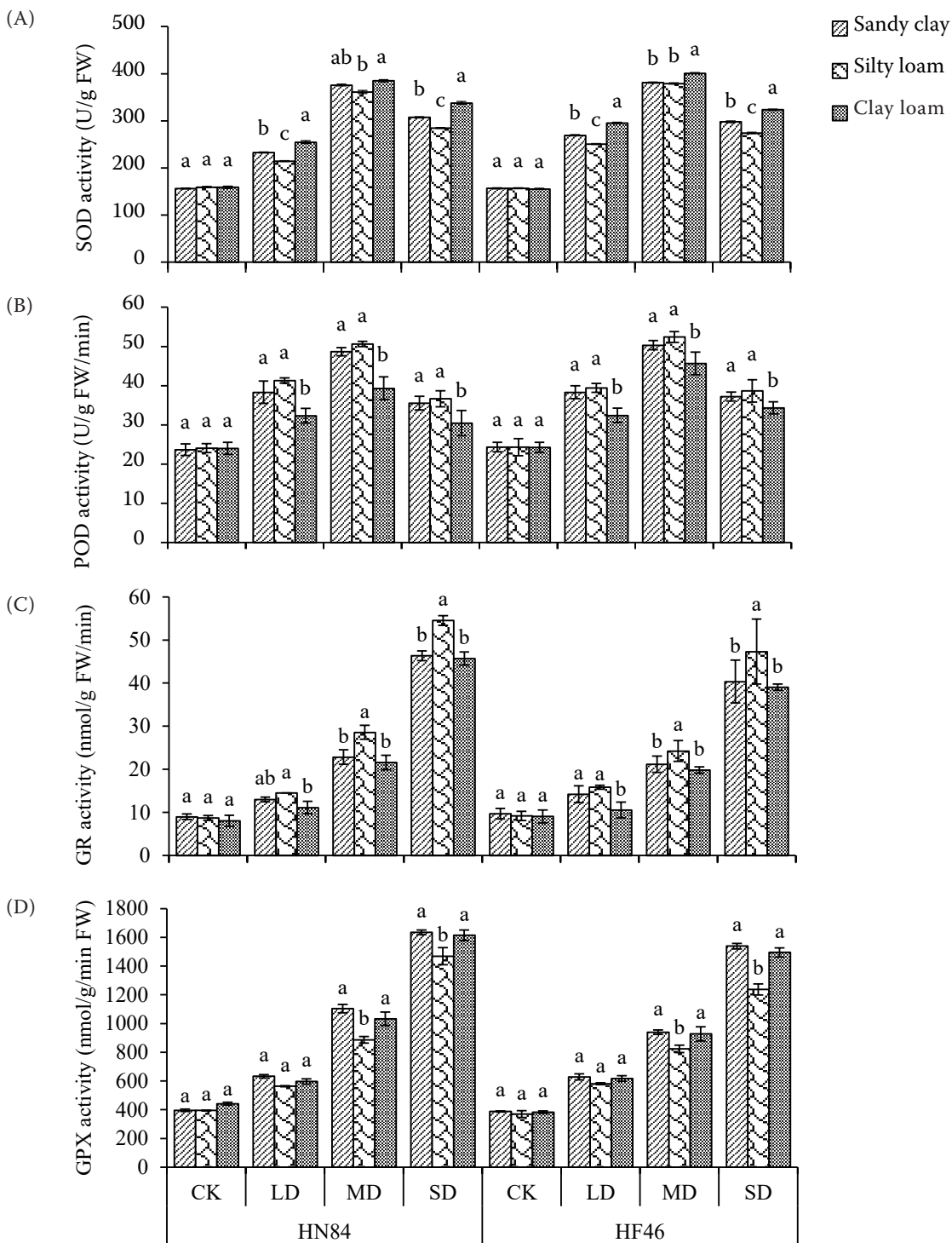


Figure 2. The activities of (A) superoxide dismutase (SOD); (B) peroxidase (POD); (C) glutathione reductase (GR), and (D) glutathione peroxidase (GPX) in soybean leaves planted in three kinds of soils under different water conditions, respectively. The level of significance is $P < 0.05$. CK – control; LD – light drought; MD – moderate drought; SD – severe drought. Error bars indicate standard error; FW – fresh weight

as POD, which was confirmed by our experimental results. We believe that the possible reason for the inconsistency with Hou’s test results is our different definitions of drought degree.

GR activities of HN84 and HF46 grown in different soils and under different water conditions are shown in Figure 2C. With the increase in drought stress, the GR activity in both soybean cultivars showed

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a trend of continuous increase, and the GR activity in soybean under SD was significantly higher than in soybean grown under other water states. The GR enzyme activity in soybean leaves was in the order of SD > MD > LD > CK, which was consistent in all three soil types. In sandy clay, silty loam, and clay loam, GR activity in HN84 leaves under SD was 416.3, 525.2, and 467.0% higher than in HN84 grown under CK conditions, respectively. Under SD conditions, GR activity in HF46 grown in sandy clay, silty loam, and clay loam was 316.4, 415.4, and 330.0% higher than CK, respectively. Under SD, the increased rate of GR activity in HN84 was higher than in HF46. Under different drought conditions, the GR activity of soybean in silty loam was significantly higher than in the other types of soil. Islam et al. (2021) suggested that the increase of antioxidant enzyme activity under drought conditions can protect plants from free radicals and reactive oxygen species to the maximum extent. Therefore, with the increasing degree of drought stress, GR activity showed a rising trend. This is consistent with our findings. Syed et al. (2021) asserted that under drought stress, SOD, POD, and GR activities increased and showed a positive correlation. In contrast, our analysis showed a positive correlation only between SOD and POD, while GR did not show a significant positive correlation with changes in SOD and POD. We hypothesise that the difference in GR-related results may be caused by the difference in the drought resistance mechanism of different cultivars and the different planting conditions.

The change of GPX activity of HN84 and HF46 in different soils and under different water conditions is shown in Figure 2D. With the increase in drought stress, the GPX activity in both soybean cultivars showed an increasing trend. The GPX activity in soybean leaves under SD was significantly higher than under other soil moisture states. The order of GR enzyme activity in soybean leaves under different soil moisture states (SD > MD > LD > CK) was consistent in all three types of soil. The GPX activity in HN84 leaves under SD was 312.8, 272.2, and 265.7% higher than under CK conditions in sandy clay, silty loam, and clay loam, respectively. Under SD, the GPX activity of HF46 was 296.4, 235.3, and 290.3% higher than under CK conditions in sandy clay, silty loam, and clay loam, respectively. Under SD, the increase of GPX activity in HN84 in sandy clay and silty loam was higher than in HF46, but the opposite was true in clay loam. Under drought stress,

the GPX activity was the lowest in plants grown in silty loam, which was significantly lower than in other soil under MD and SD conditions. Hou et al. (2021) asserted that the activities of GPX increased with the increase of drought stress in *Carex*, which is similar to our trial results.

Effects of drought stress on enzyme activity of nitrogen metabolism. Nitrate reductase is a key enzyme in nitrogen metabolism in plants. As a rate-limiting enzyme in the process of nitrogen (N) assimilation, it responds to changes in plant metabolic and physiological states. Changes in NR content can be used as an indicator of the degree of drought (Caravaca et al. 2005, May et al. 2011). The NR activity of HN84 and HF46 in different soils and under different water conditions is shown in Figure 3A. Drought stress continuously reduces the NR activity in soybean leaves, and the NR activity in soybean leaves under drought conditions is significantly lower than under CK conditions. The NR activity sequence in soybean leaves is CK > LD > MD > SD, and the three soils show approximately the same performance. The activity of NR of HN84 grown under SD conditions was lower by 80.3, 79.9, and 81.0% than in plants grown under CK in sandy clay, silty loam, and clay loam, respectively. The activity of NR of HF46 under SD was lower by 82.6, 82.1, and 83.3% than in plants grown under CK in sandy clay, silty loam, and clay loam, respectively. Under SD conditions, the decrease in NR activity in HN84 was lower than in HF46. The NR activity of soybean in sandy clay under LD and MD conditions was significantly lower than in the other two soils. Pawar et al. (2015) argued that the activities of NR showed a decreasing trend after drought stress was applied to chickpea cultivars, which is similar to our results. Except for NR, both GS and GOGAT are also important enzymes for nitrogen metabolism in plants.

Sui et al. (2021) showed that the activities of GS in *Elymus magellanicus* (Desv.) Á. Löve were higher than CK under drought stress, which was similar to our test results. But Pawar et al. (2015) argued that the activities of GS showed a decreasing trend after drought stress was applied to chickpea cultivars. In our experiment, the GS activity of HN84 and HF46 in different soils and under different water conditions is shown in Figure 3B. The GS activity of soybean leaves increased under drought stress, reaching a maximum under MD conditions and then decreasing with a further increase in drought stress. The GS activity in soybean leaves under MD conditions was

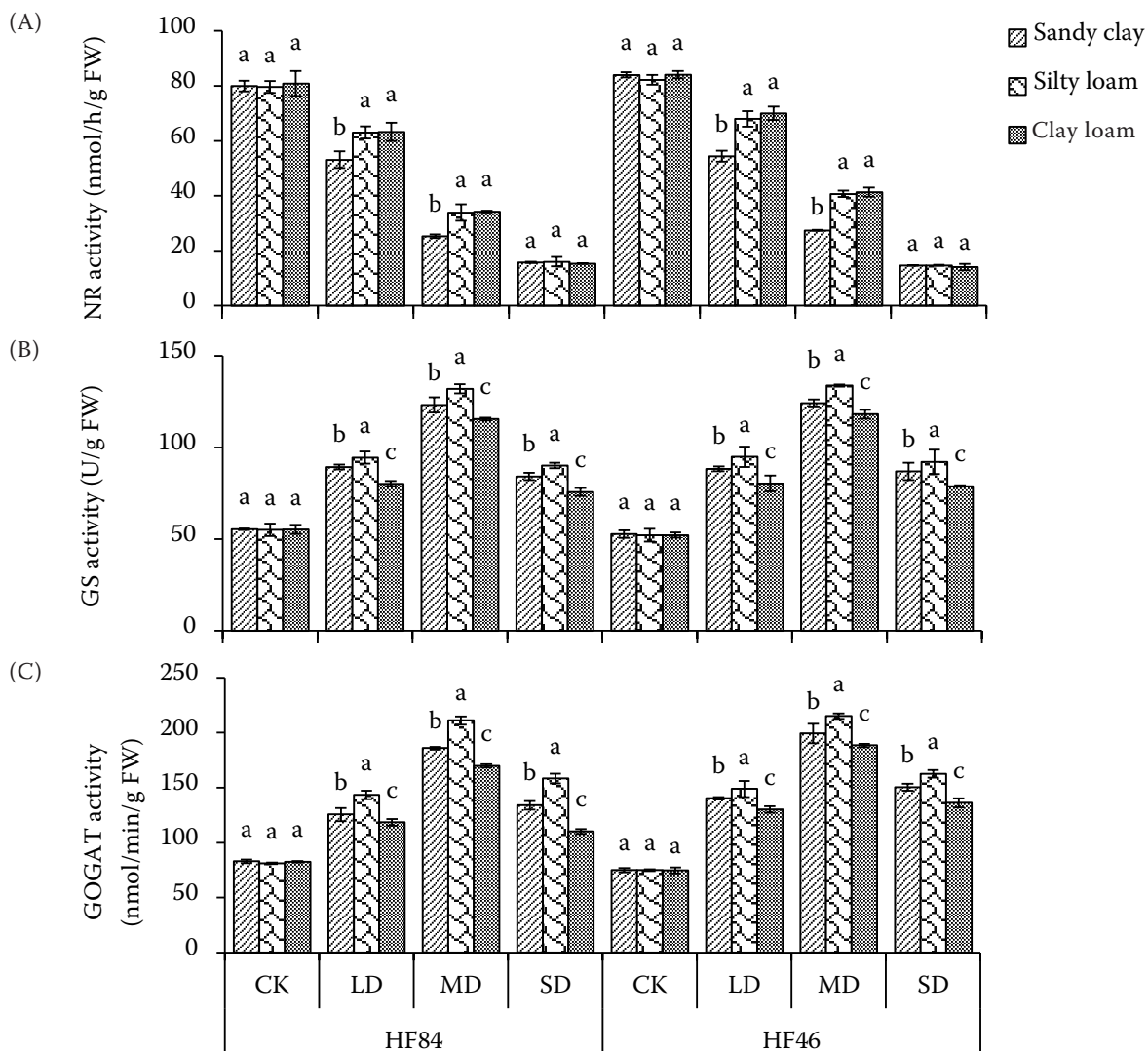


Figure 3. The activities of (A) nitrate reductase (NR); (B) glutamine synthase (GS), and (C) glutamate synthase (GOGAT) in soybean leaves planted in three kinds of soils under different water conditions, respectively. The level of significance is $P < 0.05$. CK – control; LD – light drought; MD – moderate drought; SD – severe drought. Error bars indicate standard error; FW – fresh weight

significantly higher than under other water conditions, and the order of GS activity of GS in soybean leaves under different drought stress conditions was MD > LD > SD > CK. The GS activity of HN84 under MD was higher by 122.5, 139.9, and 109.0% than in plants grown under CK conditions in sandy clay, silty loam, and clay loam, respectively. The GS activity of HF46 under MD was higher by 135.7, 156.4, and 126.5% than in plants grown under CK in sandy clay, silty loam, and clay loam, respectively. In MD, the increase in GS activity of HF46 was higher than in HN84. Under drought stress, the GS activity in silty loam was significantly higher than in sandy clay, and the GS activity in sandy clay was significantly higher

than in clay loam. Studies have shown that under short-term or mild water stress, GS and GOGAT activities increase in the short term to remove previously metabolically accumulated NH_4^+ (Zhong et al. 2018) in order to weaken or eliminate the toxic effects of NH_4^+ accumulation on plant cells (Zhao et al. 2021). However, when the stress time was too long, or the stress degree was too heavy, the activities of GS and GOGAT decreased significantly due to the limitation of NR activity (Zhao et al. 2021). It has also been suggested that the decrease in GS and GOGAT activity under severe drought or prolonged drought conditions is due to increased hydrolase activity (Cao et al. 2022) or the inhibition of the GS/GOGAT cycle

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by drought stress leading to a significant decrease in GS and GOGAT activity (Du et al. 2020).

The GOGAT activity of soybean grown in different soils and under different water conditions is shown in Figure 3C. Drought stress results in GOGAT activity in soybean leaves increasing, reaching a maximum under MD conditions, and then decreasing with a further increase in drought stress. The GOGAT activity in soybean leaves under MD conditions was significantly higher than under other water conditions, and the performance of the three soils is consistent. Under the condition of the GOGAT activity in HN84 leaves under MD was 123.9, 159.9, and 105.7% higher than in plants grown under CK conditions, respectively. The GOGAT activity of HF46 under MD was 165.7, 186.8, and 152.1% higher than in plants grown under CK conditions, in sandy clay, silty loam, and clay loam, respectively. The order of GOGAT activity in HN84 leaves grown in clay loam under different drought stress conditions was MD > LD > SD > CK. In sandy clay and silty loam, the order of GOGAT activity was MD > SD >

LD > CK. Under MD conditions, the increase of GOGAT activity HF46 was higher than HN84. The GOGAT activity in plants grown in silty loam was significantly higher than in plants grown in sandy clay, and GOGAT activity in plants grown in sandy clay was significantly higher than in plants grown in clay loam. For nitrogen metabolism, the first step is that NR and nitrite reductase (NiR) convert nitrate (NO₃⁻) into ammonia (NH₄⁺), and then GS and GOGAT assimilate NH₄⁺ into glutamic acid. NR is the rate-limiting enzyme in the process of nitrogen metabolism. When NR activity decreases under drought stress, the decrease of GS and GOGAT activity is inevitable. Pawar et al. (2015) also reached a similar conclusion when studying chickpea cultivars. However, Meng et al. (2016) believed that *Populus simonii* Carrière might enhance drought tolerance by increasing NH₄⁺ absorption and slowing down nitrogen metabolism. Soybean can be symbiotically associated with rhizobia, so we speculate that the increased activities of GS and GOGAT in soybean under drought stress might be related to rhizobia.

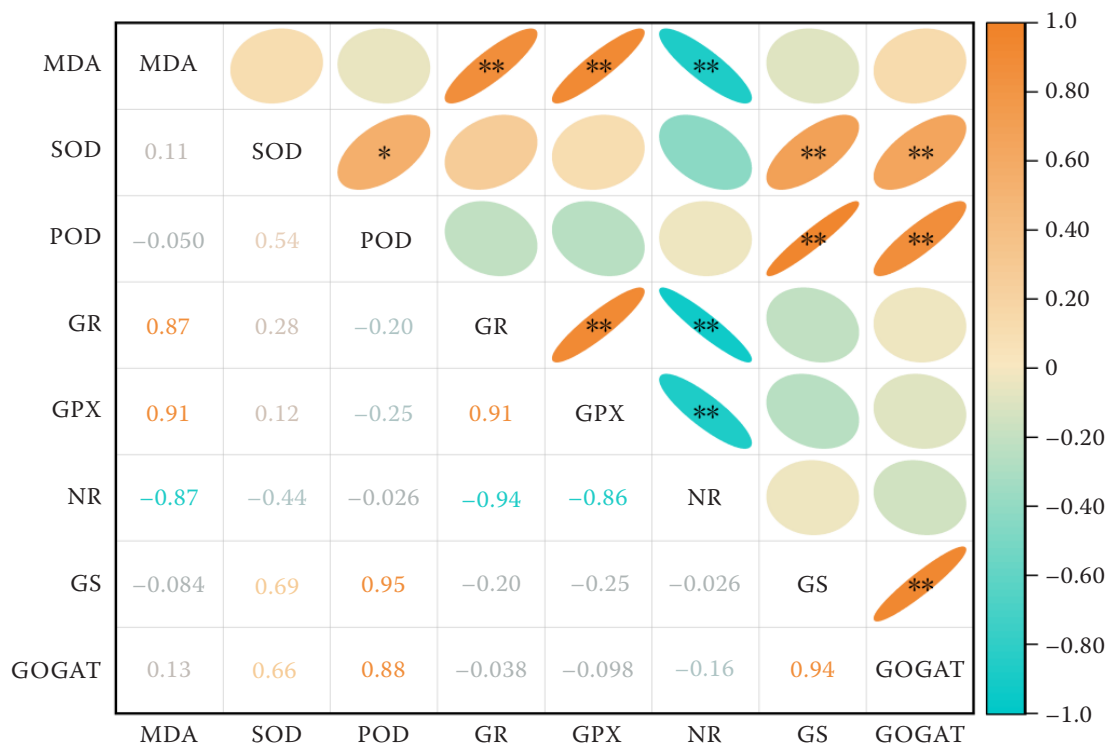


Figure 4. Correlation analysis between different physiological indicators. The correlation between eight physiological indicators is shown in the figure; the horizontal and vertical coordinates are eight different physiological indicators; close to 1 indicates a stronger positive correlation, close to -1 indicates a stronger negative correlation, and close to 0 indicates no correlation. $P \leq 0.05$; $**P \leq 0.01$ indicates extremely significant. MDA – malondialdehyde; SOD – superoxide dismutase; POD – peroxidase; GR – glutathione reductase; GPX – glutathione peroxidase; NR – nitrate reductase; GS – glutamine synthase; GOGAT – glutamate synthase

This may be a unique stress mode of soybean under drought stress, and finally, the activity of GS and GOGAT decreased due to the limitation of NR activity and drought stress (Du et al. 2020, Zhao et al. 2021).

Correlation analysis of changes of different physiological indexes. We conducted correlation analysis on the changes in eight physiological indexes studied in this test after drought stress (Figure 4) and found that SOD and POD were significantly positively correlated, GR and GPX were extremely significantly positively correlated, and SOD and POD with GR and GPX were not significantly correlated. There was a significant positive correlation between GS and GOGAT but no significant correlation between GS and GOGAT with NR among nitrogen metabolism enzymes. In general, the changes in SOD, POD, GS, and GOGAT were positively correlated. The changes in MDA, GR, and GPX were positively correlated. The levels of NR were negatively correlated with GPX, GR, and MDA. Hou et al. (2021) asserted that the activities of POD and GPX increased with the increase of drought stress in *Carex*, while the activity of SOD decreased first and then increased. In our experiment, the activity of POD increased first and then decreased, which may be caused by the difference in drought stress degree, stress time, and species used in the test.

One of the objectives of this study was to determine whether there were significant physiological differences in soybean planted under the same drought conditions and different soil conditions. Due to the difference in soil quality itself, it is likely to cause different physiological responses under drought stress, which has been demonstrated in *Gardenia jasminoides* J. Ellis and Chinese cabbage. According to Luo (2021), gardenia was tested under drought conditions in two soil substrates, and it was found that there were significant differences in MDA content and antioxidant enzyme activity in gardenia suffering drought stress grown in loess substrate and purple soil substrate. Lurong et al. (2021) showed that the physiological conditions of Chinese cabbage, such as SOD, POD, and MDA, vary greatly in different soils and under different drought stress conditions, which was similar to our experimental results. Therefore, we believed that soil conditions played an important role in the growth of crops. For different soybean cultivars and different planting soils, the response to drought stress will also be different. In general, HN84 is slightly more resistant to drought than HF46, and the drought

tolerance of clay loam was the strongest, and that of silty loam was the weakest under the three different drought conditions. The relatively high clay content in clay loam gives it high water retention. Under the same conditions of relative water content, the moisture available for plants in clay loam is higher than in silty loam. This results in a higher degree of drought stress for plants grown under the same relative moisture conditions in silty loam. The physiological indices of soybean planted in silty loam under drought stress changed the most, which is a result of the nature of the soil itself. Correlation between different physiological indices of soybean under drought conditions is related to resistance mechanisms of soybean grown under drought stress. Our experiment provides a theoretical basis for further study on the suitability of different soils for soybean growth and the stress resistance mechanisms of soybean under drought stress.

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