LRF-816
[1-7]

RESEARCH ARTICLE Legume Research- An International Journal

Effects of Drought Stress on Key Enzymes in Soybean Carbon and Nitrogen Metabolism

Z.P. Qu[1], X.Y. Zhou[1], C.X. Liu[1], X.Y. Wang[1], S.K. Dong[1]

10.18805/LRF-816

ABSTRACT

Background: The synthesis and catalysis of enzymes are inherently linked to the presence of water and drought-induced water deficit in plants can adversely impact enzyme activity. Drought severely impairs the growth and development of crops, with the extent of its impact varying according to the severity of the water scarcity. Drought also regulates the activity of carbon and nitrogen metabolizing enzymes in plants.

Methods: To investigate the effects of varying degrees of drought on soybeans, this study selected HN44 (drought-resistant variety) and HN65 (drought-sensitive variety) as subjects through sand culture and assessed the changes in key enzymes involved in carbon and nitrogen metabolism within the soybean leaves in 2022.

Result: The results indicated that short-term drought stress increased the activities of sucrose synthase (SUS), Sucrose Phosphate Synthase (SPS) and Glutamine Synthetase (GS), while decreasing the activity of nitrate reductase (NR). In contrast, prolonged drought reduced the activities of SUS and GS. This study provides a theoretical foundation for enhancing the drought stress resistance of soybeans.

Key words: Aridity gradient, Carbon metabolism, Legumes study, Nitrogen metabolism

INTRODUCTION

Soybean [Glycine max (L.) Merr]. is one of the primary oil-bearing crops in Northeast China (Konno and Homma, 2023). The soybean production in the Northeast region of China in 2022 accounted for over 50% of the national total (Di *et al*., 2023) and spring drought has become a significant factor limiting the soybean yield in the Northeast region (Santini *et al*., 2022). Furthermore, in the event of spring drought, soybeans are at the seedling stage of growth and varying degrees of drought had distinct impacts on soybean's carbon and nitrogen metabolism. Consequently, investigating the effects of different levels of drought on soybean's carbon and nitrogen metabolism has become a focal point of research (Zhou *et al*., 2022).

Under the duress of drought, plants mitigate water loss by constricting their stomata, thereby diminishing transpiration (Patel *et al*., 2022). Yet, this stomatal closure concurrently impedes the plant's capacity to diffusion carbon dioxide from the atmosphere-a critical component for photosynthesis-resulting in a state of carbon deficiency within the plant (Alyemeni and Al-Quwaiz, 2014). Therefore, key enzymes involved in carbon metabolism have become the focus of research in such circumstances. In the carbon metabolic pathway of soybeans, two pivotal enzymes play a crucial role: sucrose synthase (SUS) and sucrose phosphate synthase (SPS) (Song *et al*., 2022). The influence of drought on soybean cultivation is particularly evident in the activities of key enzymes SUS and SPS, which are integral to the plant's carbon metabolism. Xing *et al.* (2018) examined how drought conditions during the flowering period affect the carbon metabolism in soybeans. Their findings revealed a significant enhancement in the enzymatic activities of SPS and SUS under drought stress.

[1]College of Agriculture, Northeast Agricultural University, Harbin, China.

Corresponding Author: S.K. Dong, College of Agriculture, Northeast Agricultural University, Harbin, China.
Email: shoukundong@163.com

How to cite this article: Qu, Z.P., Zhou, X.Y., Liu, C.X., Wang, X.Y. and Dong, S.K. (2024). Effects of Drought Stress on Key Enzymes in Soybean Carbon and Nitrogen Metabolism. Legume Research. DOI: 10.18805/LRF-816.

Submitted: 24-04-2024 **Accepted:** 09-07-2024 **Online**: 31-07-2024

Nitrogen is an essential nutrient for the construction of plant tissues and numerous studies have indicated a correlation between nitrogen metabolism and plant stress resistance (Wang *et al*., 2023). Nitrogen metabolism involves key enzymes such as nitrate reductase (NR) and Glutamine Synthetase (GS), which play pivotal roles in the biochemical processes that enable plants to adapt and thrive in various environmental conditions (Dai *et al*., 2022). SUS and SPS promote the conversion of carbon, NR and GS facilitate the assimilation of nitrate and ammonium, which are crucial pathways for plants to incorporate these carbon and nitrogen forms into their metabolic networks, thereby sustaining growth and development (Li *et al*., 2015).

The impact of varying degrees of drought on the carbon and nitrogen metabolism of soybeans remains an open question (Kimball *et al*., 2016). Therefore, sand culture method was used in this experiment to better control different drought gradients and nutritional conditions of plants. In this experiment, using the drought-resistant type Heinong 44 (HN44) and drought-sensitive type Hei-nong 65 (HN65) as experiment materials. By irrigating with different concentrations of PEG-6000, this study aims to investigate the effects of different levels of drought stress on the carbon and nitrogen metabolism in soybeans. This study aims to further understand the changes of carbon and nitrogen metabolism of soybean in different drought-sensitive populations under drought stress and provide theoretical support for subsequent identification and screening of drought-tolerant seed resources.

MATERIALS AND METHODS

The experimental varieties are HN44 and HN65. Based on the drought resistance screening test results of Wang *et al.* (2012) HN44 is identified as a drought-resistant variety, while HN65 is classified as a drought sensitive variety.

The experiment was conducted at the campus of Northeast Agricultural University in 2022. A sand culture potting method was employed, utilizing plastic buckets with a diameter of 30 cm and a height of 30 cm. To enhance the drainage and aeration of the buckets, four 1-centimeter diameter holes were drilled at the bottom and a piece of mesh was placed inside. Subsequently, the buckets were filled with washed river sand and thoroughly watered. Selected soybean seeds with plump and uniform size, free from diseases, were used for sowing, with eight seeds per bucket. After sowing, a 1 cm layer of dry sand was spread on the surface. From sowing until the complete expansion of the first pair of true leaves, the plants were irrigated daily with 500 mL of clear water. Thinning was conducted when the true leaves had fully expanded, ensuring that four seedlings were retained per bucket. Subsequently, the seedlings were irrigated daily with a nutrient solution (Dong *et al.*, 2023), at a rate of 500 mL per application. Drought stress treatment was initiated when the seedlings reached the V3 growth stage. Different amounts of PEG-6000ÿmacromolecular substances commonly used to simulate drought stressÿ were added to the nutrient solution to create nutrient solutions with concentrations of 5%, 10%, 15% and 20%. These solutions were used to simulate varying degrees of drought stress by irrigating the plants once daily with 500 mL of the respective solution. A control group (CK) was irrigated with a nutrient solution lacking PEG-6000 at the same frequency and volume as described above. Nutrient solution configuration refers to Dong *et al.* (2023). After the implementation of drought stress, samples were collected every three days for a total of six times. Sampling was conducted between 8:00 and 9:00 AM, targeting the second-to-last leaf of the plant. Three pots were selected for each sampling event, with each pot serving as an individual replicate. The collected leaves were divided into two parts. The first part was preserved in an ice box to maintain freshness and was immediately subjected to measurements (NR, GS); the second part was stored by rapid freezing in liquid nitrogen for subsequent analysis (SUS, SPS).

The assay method for SUS can be referenced from the method by Chengappa *et al.* (1999). Weigh 0.1g of soybean leaf tissue and place it into a precooled mortar. Add 1ml of buffer solution and grind thoroughly. Following this, centrifuge the mixture at 10000g for 20 minutes at 4°C and collect the 90µL of enzyme solution. Combine this with 110µL of the reaction mixture (consisting of 50µL of HEPES-NaOH, 20µL of 50mmol/L MgCl2, 20µL of 100mmol/L fructose, and 20µL of 100mmol/L UDPG). Incubate the reaction at 30°C for 10 minutes.

The activity assay for SPS is similar to that of SUS, with the exception that in the reaction system, 20 µL of 100 mmol/L fructose is replaced with 20 µL of 100 mmol/L fructose-6-phosphate.

The assay method for GR can be referenced from the method by Vezina *et al.* (1989). Take 0.1 g of soybean leaf tissue and place it into a mortar. Add 0.5 ml of extraction solution to the mortar. Grind the leaf tissue and extraction solution together in an ice bath until a homogeneous paste is formed. Transfer the ground mixture to a centrifuge tube and centrifuge at 15,000g for 20 minutes at 4°C. Take 0.1 ml of Reaction B solution, 0.05 ml of crude enzyme solution and 0.05 ml of ATP solution and mix them evenly in a new centrifuge tube. Place the centrifuge tube containing the reaction mixture into a water bath at 37°C and incubate for 30 minutes. Add 0.1ml of color reagent to the reaction mixture. Let the mixture stand at room temperature for 5 minutes to complete the color development. Centrifuge the colored mixture at 33,500 rpm for 10 minutes to separate the supernatant from the precipitate. Take the supernatant for subsequent measurement and analysis.

NR Activity Measurement: Weigh out 1 gram of fresh sample material and place it into a beaker. Press the material firmly into the bottom of the beaker using an object to ensure it is well compacted. To a control beaker, add 1 ml of trichloroacetic acid (TCA) at a concentration of 0.3 grams per millilitre. To the treatment group beaker, add 9 ml of potassium nitrate (KNO_3) solution at a concentration of 0.1 mol/L. Proceed with the subsequent steps according to the method described by Qu *et al.* (2023).

Microsoft Office 365 (Home edition) for writing. Microsoft Excel (2010) was used to calculate the deviation and the calculation function was stdev.P. IBM SPSS Statistics 27.0.1 (IBM Corporation, Armonk, NY, USA) performed significance analysis (Tukey's HSD test). All significance analyses were comparisons between different treatments of the same variety on the same day.

RESULTS AND DISCUSSION

Effects of different degrees of drought on SUS activity of soybean

Table 1 shows the changes in the activity of Sucrose Synthase (SUS) in the leaves of the HN44 and HN65 under different levels of drought stress. Under different levels of drought conditions, the activity of SUS in both varieties shows a trend of first increasing and then decreasing with the extension of stress time, with the peak value occurring at 9 days or 12 days. Moreover, in the early stages of drought, more severe drought conditions will lead to a higher activity of SUS compared to the CK.

In HN44, the activity of SUS under drought stress significantly increased compared to the CK at 3 days and 6 days for treatments 10%, 15% and 20% by 19.62%, 20.88% and 32.27%, respectively, as well as by 7.50%, 9.69% and 12.18%. At the 15 days, treatment 20% was significantly lower than the other treatments by 13.35%, 10.08% and 11.99%, respectively and by 5.99% compared to CK. (Table 1).

In HN65, the activity of SUS significantly increased with the 20% treatment under drought stress at 3 days, showing an 18.62% rise compared to the CK. Under drought stress for 15 days and 18d, the remaining treatments were all significantly lower than CK, with reductions of 5.19%, 7.31%, 10.85%, 15.57%, 15.40%, 19.91%, 20.14% and 24.41%. (Table 1).

The results indicate that with increasing severity of drought, the decline in SUS activity is more pronounced, with HN44 exhibiting a smaller decrease in activity compared to HN65. Specifically, across all treatments, HN44 saw a decrease in SUS activity by 7.04%, 7.75%, 12.91% and 16.90%, while HN65 experienced a decline of 15.40%, 19.90%, 20.14% and 24.41%. In this study, HN44 exhibited higher SUS activity than CK at 3 and 6 days of stress, while HN65 only showed higher SUS activity than CK at 3 days of drought. This suggests that HN44 has a greater capacity for sucrose synthesis during the initial stages of stress, which may enable it to produce more substances for drought regulation. However, when the duration of drought is prolonged, the sucrose synthesis capacity of both varieties is suppressed, yet HN44 still demonstrates relatively higher sucrose synthesis ability (Table 1). Aliche *et al*. (2020) investigated the impact of drought stress on potato carbon metabolism and observed an increase in SUS activity under short-term drought, aligning with the findings of this study.

Effects of different degrees of drought on SPS activity of soybean

Table 2 presents the changes in the activity of SPS in the leaves of HN44 and HN65 under varying degrees of drought stress. For both varieties, the SPS activity under treatment

Table 1: Effects of different degrees of drought on SUS activity of soybean leaves (mg/g FW/min).

Varieties	Treatment	3 days	6 days	9 days	12 days	15 days	18 days
HN44	CK	1.58±0.03a	3.2±0.09a	4.1±0.1a	4.17±0.1a	4.16±0.06a	4.26±0.13a
	5%	1.66±0.07a	3.2±0.05a	4.16±0.1a	4.26±0.04a	4.04±0.02a	3.96±0.09b
	10%	1.89±0.13b	3.44±0.11b	4.14±0.08a	4.14±0.01a	4.11±0.07a	3.93±0.09b
	15%	1.91±0.04b	3.51±0.05b	4.21±0.02a	4.12±0.02ab	3.89±0.02b	3.71±0.09bc
	20%	2.09±0.03b	3.59±0.03b	4.07±0.06a	3.93±0.08b	3.67±0.02c	3.54±0.03c
HN650	CK	1.88±0.18a	3.19±0.04a	4.21±0.02a	4.27±0.02a	4.24±0.03a	4.22±0.04a
	5%	2.03±0.03ab	3.07±0.11a	4.31±0.04a	4.16±0.04b	4.02±0.01b	3.57±0.02b
	10%	2.05±0.04ab	3.08±0.17a	4.2±0.03a	4.07±0.05b	3.93±0.15b	3.38±0.04c
	15%	2.19±0.05ab	3.14±0.02a	4.27±0.04a	4.03±0.06bc	3.78±0.07b	3.37±0.06c
	20%	2.23±0.09b	3.1±0.07a	3.99±0.01b	3.92±0.05c	3.58±0.02c	3.19±0.08d

Longitudinal comparison within each variety, Using one-way analysis of variance method. Different letters indicate that each line is significantly different at the 5% level, CK: Normal water. The different numbers in the column of treatments indicate the proportion of mixing with water (kg/100L).

Table 2: Effects of different degrees of drought on SPS activity of soybean leaves (mg/g FW/h).

Varieties	Treatment	3 days	6 days	9 days	12 days	15 days	18 days
HN44	CK	4.36±0.17a	4.33±0.05a	4.48±0.28a	5.09±0.26a	5.22±0.20a	5.14±0.38a
	5%	4.52±0.26a	4.63±0.23a	6.91±0.17ab	11.69±0.29b	16.23±0.85b	22.58±0.48d
	10%	5.82±0.30b	5.76±0.15b	8.16±0.15b	16.44±2.07c	24.3±2.23c	18.12±0.47c
	15%	7.72±0.55c	10.8±0.05c	17.5±1.22c	34.12±0.31d	22.54±1.33c	15.6±0.99b
	20%	9.18±0.22d	12.94±0.06d	21.41±1.88d	35.15±1.31d	23.29±0.82c	14.29±0.70b
HN650	CK	5.61±0.17a	6.00±0.24a	6.80±0.26a	6.92±0.28a	6.47±0.57a	6.71±0.23a
	5%	8.06±0.20b	10.85±0.16b	11.02±0.34b	13.23±1.06b	16.56±0.87b	21.04±1.83d
	10%	9.13±0.16c	11.89±0.15c	16.89±1.30c	21.9±0.89c	16.25±0.82b	14.78±0.31c
	15%	10.87±0.2d	16.79±0.1d	23.15±1.21d	33.62±1.37d	23.57±0.2c	11.54±0.54b
	20%	12.25±0.16e	22.19±0.52e	27.1±0.88e	35.22±0.76d	22.38±0.79c	7.79±0.31a

Longitudinal comparison within each variety, Using one-way analysis of variance method. Different letters indicate that each line is significantly different at the 5% level, CK: Normal water. The different numbers in the column of treatments indicate the proportion of mixing with water (kg/100L).

5% exhibited a continuous increase with the prolongation of stress time. For the other treatments, the SPS activity initially increased and then decreased, with the peak activity occurring on the 12 or 15 day.

In the HN44 variety, during the stress period from 3 days to 9 days, except for the 5% treatment which showed no significant difference compared to the CK, the SPS activity increased more significantly with higher levels of drought stress in the other treatments. Specifically, at 9 days, the SPS activity in the remaining treatments increased by 54.24%, 82.14%, 290.63% and 377.90% respectively compared to the control. At 18 days, the SPS activity in treatments 15% and 20% was significantly lower than that in 10% and all three treatments were markedly lower than the 5% treatment (Table 2).

In the HN65 soybean variety, the activity of SPS exhibited a pronounced upward trend in response to escalating drought stress, ranging from 3 to 9 days. At the 9 days, the SPS activity, when contrasted with the CK, witnessed a substantial rise of 62.06%, 148.38%, 240.44% and 298.53% across the different treatments. As the drought stress persisted to the 15 days, treatments 15% and 20% continued to maintain significantly elevated SPS activity levels compared to 5% and 10%. (Table 2).

These findings suggest that soybeans utilize the SPS pathway to synthesize sucrose during periods of drought, which in turn allows them to accumulate additional resources for coping with water scarcity. Notably, the HN44 variety demonstrated a more pronounced elevation in SPS activity compared to HN65, indicating a superior capacity for sucrose metabolism, which could be a key factor in its enhanced drought tolerance. The activity of SPS increases with the severity of the drought, with the peak activity rising more significantly as the drought becomes more severe. However, this pathway is noticeably inhibited as the duration of stress extends and the higher the degree of drought, the earlier this inhibition occurs (Table 2). Xing *et al*. (2018) and others conducted drought treatment on soybeans during the flowering stage and observed a significant increase in SPS activity.

Table 3: Effects of different degrees of drought on NR activity of soybean leaves (ìg/g FW/h).

Varieties	Treatment	3 days	6 days	9 days	12 days	15 days	18 days
HN44	CK	45.14±2.17a	45.14±1.22a	44.54±1.25a	48.86±3.15a	51.46±1.61a	53.05±2.38a
	5%	44.9±2.22a	39.97±2.07b	37.99±1.69b	37.39±1.23b	34.88±0.81b	22.50±2.99b
	10%	42.09±1.50a	39.52±0.14b	32.41±1.10c	29.67±1.28c	25.16±4.35c	20.11±1.35b
	15%	39.92±1.17a	32.92±2.02c	26.2±2.64d	22.26±0.68d	14.32±2.31d	5.26±0.16c
	20%	34.43±0.86b	29.84±1.74c	22.07±2.08d	16.84±1.84d	9.22±0.10d	4.88±0.20c
HN65	CK	35.48±1.61a	36.28±0.46a	39.56±0.26a	42.01±0.77a	41.43±0.58a	44.33±1.04a
	5%	34.90±1.98a	32.67±1.16b	31.62±0.16b	30.48±4.27b	26.94±0.81b	13.49±0.48b
	10%	34.07±2.14a	31.03±0.14b	29.50±0.34b	26.05±2.44b	22.01±0.16c	6.39±0.50c
	15%	30.07±1.50ab	27.94±1.36c	25.50±1.00c	19.96±2.95c	5.66±0.25d	4.98±0.18c
	20%	27.84±1.35b	25.77±0.51d	23.45±0.24d	16.86±3.65c	6.28±0.37d	4.79±0.08c

Longitudinal comparison within each variety, Using one-way analysis of variance method. Different letters indicate that each line is significantly different at the 5% level, CK: Normal water. The different numbers in the column of treatments indicate the proportion of mixing with water (kg/100L).

Table 4: Effects of different degrees of drought on GS activity of soybean leaves (A540/g FW/h).

Varieties	Treatment	3 days	6 days	9 days	12 days	15 days	18 days
HN44	CK	77.71±1.95a	80.77±5.58a	120.57±1.19a	170.74±1.96a	156.67±10.1a	156.21±7.41a
	5%	80.97±2.61ab	94.12±1.71b	137.11±3.56b	143.36±2.21b	133.78±3.72b	108.14±3.99b
	10%	78.50±2.40ab	101.17±1.68b	149.58±2.31b	141.83±6.75b	119.27±5.62b	84.52±3.32c
	15%	78.3±2.75ab	121.07±1.78c	151.48±5.59b	111.82±0.80c	89.19±5.16c	70.94±2.23d
	20%	86.93±3.39b	133.58±3.41d	167.54±5.97c	99.55±7.69c	79.06±2.53c	59.31±1.79d
HN650	CK	36.59±0.38a	49.99±3.73a	65.8±3.56a	93.3±3.86a	92.23±1.14a	97.76±1.05a
	5%	38.39±0.28a	61.95±2.11b	82.58±1.1b	86.18±3.04a	59.78±1.43b	54.30±1.05b
	10%	42.68±3.33a	63.01±1.78b	86.99±1.75b	85.99±1.76a	53.26±2.39c	48.65±0.97c
	15%	60.07±0.98b	74.43±3.51c	95.82±1.43c	83.87±3.09ab	53.95±0.34c	41.17±2.29d
	20%	63.05±3.70b	79.55±1.83c	98.27±1.45c	74.13±4.01b	44.26±0.83d	38.35±1.77d

Longitudinal comparison within each variety, Using one-way analysis of variance method. Different letters indicate that each line is significantly different at the 5% level, CK: Normal water. The different numbers in the column of treatments indicate the proportion of mixing with water (kg/100L).

Effects of different degrees of drought on NR activity of soybean

Table 3 illustrates the fluctuation in NR activity within the leaves of the HN44 and HN65 plant varieties when subjected to varying levels of drought stress. As the plants were exposed to drought stress of increasing severity, there was a notable downward trend in leaf NR activity, correlating with both the duration and the severity of the stress applied.

In the HN44 variety, NR activity experienced a notable decline specifically in the 20% treatment after 3 days of drought stress, with a decrease of 23.73% when compared to the CK. By the 6 days, NR activity in all treatments had significantly decreased relative to CK, with reductions ranging from 11.54% to 33.90%. Notably, the 15% and 20% treatments demonstrated significantly lower NR activity levels than the 5% and 10% treatments. By the 18th day, the NR activity in the 15% and 20% treatments were again significantly lower than in the 5% and 10% treatments (Table 3).

In the HN65 variety, NR activity saw a significant drop exclusively in the 20% treatment after 3 days of drought stress, with a 21.53% decrease from the CK. By day 15, there was no significant variation between the 15% and 20% treatments, but all other treatments demonstrated significant effects. On day 18, treatments 10%, 15% and 20% were all markedly lower than 5%, with respective decreases of 52.63%, 63.08% and 64.49%, lower than the 5% treatment, with reductions of 52.63%, 63.08% and 64.49% (Table 3).

The NR activity of HN44 under various treatments decreased by 57.59%, 62.09%, 90.08% and 90.80% compared to the CK, respectively. In contrast, HN65 exhibited a decline of 69.57%, 85.59%, 88.77% and 98.31% under the same conditions. The results indicate that the higher the degree of drought, the greater the decrease in NR activity. When subjected to milder drought conditions, HN44 showed a smaller decline in NR activity compared to HN65. However, under more severe drought conditions, the decline in NR activity for HN44 was almost identical to that of the sensitive varieties. Notably, the variations in HN44, both in terms of increase and decrease, are consistently less pronounced than those observed in HN65 (Table 3). He *et al*. (2022) conducted research on cotton under drought stress and found that drought reduced the nitrate content in the leaves, leading to a decline in NR activity. Pandey *et al.* (2022) subjected Cyamopsis *tetragonoloba* to drought stress and observed a reduction in the activity of NR, a result that aligns with the outcomes of our current study.

Effects of different degrees of drought on GS activity of soybean

Table 4 presents the changes in the activity of the leaf's GS enzyme in HN44 and HN65 under varying degrees of drought stress. Under different levels of drought stress, the GS activity of both varieties exhibited a trend of initially increasing and then decreasing with the extension of stress duration, with the peak activity observed around the 9th or 12th day.

In HN44, the GS activity was significantly higher in the 20% treatment compared to the CK by 11.86% after 3 days of stress. At 6 days, drought stress exhibited a positive correlation with GS activity, resulting in respective increases of 16.53%, 25.26%, 49.90% and 65.38% compared to CK. At 9 days, there were no significant differences among the 5%, 10% and 15% treatments, but all were significantly lower than the 20% treatment. By day18, GS activity decreased significantly as drought severity increased (Table 4).

In HN65, the GS activity significantly increased by 64.17% for the 15% treatment and by 72.32% for the 20% treatment after 3 days of stress. At 6 and 9 days, different drought treatments significantly affected GS activity. By 12 days, the GS activity for all treatments had decreased compared to CK by 7.63%, 7.83%, 10.11% and 20.55%. By 18days, GS activity decreased gradually with the increase of drought stress degree. (Table 4).

The peak GS activity values for various treatments in HN44 increased by 13.72%, 24.06%, 25.64% and 38.96% compared to the CK, while the lowest values decreased by 30.78%, 45.90%, 54.59% and 62.03%, respectively. For HN65, the peak GS activity values increased by 25.50%, 32.20%, 45.62% and 49.35% and the lowest values decreased by 65.24%, 68.86%, 73.64% and 75.46%. The results suggest that in the early stages of drought stress, there is a certain stimulatory effect on GS activity. However, as the duration of stress increases, drought inhibits the activity of GS. Our research demonstrates that across all treatments, GS activity experiences an initial decline, which culminates in a progressive weakening as the severity of the drought escalates (Table 4). In their study on Phyllostachys *edulis*, Shi, *et al*. (2020) applied drought stress and observed that it suppressed the activity of crucial enzymes involved in nitrogen metabolism.

CONCLUSION

In the context of mild drought stress (5%), researchers observed an upsurge in the activity of SPS. In contrast, both moderate and severe drought conditions led to a dualistic response in SPS activity, characterized by an initial increase followed by a subsequent decrease. Similarly, the activities of SUS and GS exhibited a biphasic trend, with an initial rise and a subsequent decline, while the NR activity was observed to consistently decrease. Among the soybean varieties examined, which differ in their sensitivity to drought, HN44 consistently displayed higher activities of NR and GS compared to HN65. Notably, at the initial stages of drought, HN65 exhibited higher activities of SUS and SPS than HN44. However, as the period of drought continued to lengthen, the activities of SUS and SPS in HN44 gradually overtook those observed in HN65.

Through the comprehensive analysis of the activities of SUS, SPS, NR and GS, this study offers valuable theoretical references for developing strategies to improve soybean's resilience against drought stress, thereby equipping the crop with a more robust defence mechanism against water scarcity.

ACKNOWLEDGEMENT

This work was supported by the National Key Research and Development Program of China, (2020YFD1000902-2). Heilongjiang Provincial Natural Science Foundation of China (LH2021C023). The fund comes from the Heilongjiang Provincial Department of Science and Technology.

Conflict of interest

All authors declare that they no conflict of interest.

REFERENCES

Aliche, E.B., Theeuwen, T., Oortwijn, M., Visser, R.G.F. and van der Linden, C.G. (2020). Carbon partitioning mechanisms in POTATO under drought stress. Plant Physiology and Biochemistry, 146, 211-219. Retrieved from //WOS:000 510528300019. doi: 10.1016/j.plaphy.2019.11.019.

Alyemeni, M.N. and Al-Quwaiz, S.M. (2014). Effect of 28-homobrassinolide on the drought induced changes in the seeds of *Vigna radiata*. Legume Research. 37(5): 515-519. Retrieved from //WOS:000 343780800011. doi: 10.5958/0976-0571.2014.00668.7.

Chengappa, S., Guilleroux, M., Phillips, W. and Shields, R. (1999). Transgenic tomato plants with decreased sucrose synthase are unaltered in starch and sugar accumulation in the fruit. Plant Molecular Biology. 40(2): 213-221. Retrieved from Medline:10412901. doi: 10.1023/a: 10061 36524725.

Dai, X., Liu, N., Xia, Y., Yang, L., Hao, P., Dong, Z. and Che, Z. (2022). Effects of nitrogen regulation on nitrogen assimilation process and yield of maize. Journal of Anhui Agricultural University. 49(4): 533-539. Retrieved from//CSCD: 7318310.

Di, Y., You, N., Dong, J., Liao, X., Song, K. and Fu, P. (2023). Recent soybean subsidy policy did not revitalize but stabilize the soybean planting areas in Northeast China. European Journal of Agronomy.

Dong, S.K., Wang, X., Li, X.M., Tian, Y.M., Zhou, X.Y., Qu, Z.P., Liu, L.J. (2023). Mechanism of mepiquat chloride rgulating soybean response to drought stress revealed by proteomics. Plants-Basel. 12(10): Retrieved from ://WOS:0009979 69100001. doi: 10.3390/plants12102037.

He, J.Q., Hu, W., Li, Y.X., Zhu, H.H., Zou, J., Wang, Y.H., Zhou, Z.G. (2022). Prolonged drought affects the interaction of carbon and nitrogen metabolism in root and shoot of cotton. Environmental and Experimental Botany, 197. Retrieved from ://WOS:000788719400003. doi: 10.1016/ j.envexpbot.2022.104839.

Kimball, B.A. (2016). Crop responses to elevated CO₂ and interactions with H₂O, N and temperature. Current Opinion in Plant Biology. 31: 36-43. Retrieved from://WOS:000379103100007. doi: 10.1016/j.pbi.2016. 03.006.

Konno, T. and Homma, K. (2023). Prediction of Soybean Lodging by Main Stem Elongation Model. Abstracts of Meeting of the CSSJ. 47-47.

Li, W.W., Chen, M., Zhong, L., Liu, J.M., Xu, Z.S., Li, L.C., Ma, Y.Z. (2015). Overexpression of the autophagy-related gene SiATG8a from foxtail millet (*Setaria italica* L.) confers tolerance to both nitrogen starvation and drought stress in Arabidopsis. Biochemical and Biophysical Research Communications. 468(4): 800-806. Retrieved from ://WOS:000367868500045. doi: 10.1016/j.bbrc. 2015.11.035.

Pandey, K., Kumar, R.S., Prasad, P., Sushma, Pande, V., Trivedi, P.K. and Shirke, P.A. (2022). Synchronised interaction of carbon and nitrogen provides drought tolerance in Cyamopsis tetragonoloba. Environmental and Experimental Botany, 199. Retrieved from://WOS:00080628 5500004. doi: 10.1016/j.envexpbot. 2022.104899.

Patel, S.R., Th, A.S., Kumar, S. and Fougat, R.S. (2022). Evaluation of cluster bean *Cyamopsis tetragonoloba* (L.) Taub genotypes for drought stress adaptation and its effects on yield. Legume Research. 45(5): 551-556. Retrieved from ://WOS:000798367100004. doi: 10. 18805/lr-4654.

Qu, Z.P., Tian, Y.M., Zhou, X.Y., Li, X.M., Zhou, Q., Wang, X.Y. and Dong, S.K. (2023). Effects of exogenous sodium nitroprusside spraying on physiological characteristics of soybean leaves at the flowering stage under drought stress. Plants-Basel. 12(8): Retrieved from ://WOS:00097 9002000001. doi: 10.3390/plants12081598.

Santini, M., Noce, S., Antonelli, M. and Caporaso, L. (2022). Complex drought patterns robustly explain global yield loss for major crops. Scientific Reports. 12(1): Retrieved from //WOS:0007801.

Shi, W.H., Lin, L., Shao, S.L., He, A.G. and Ying, Y.Q. (2020). Effects of simulated nitrogen deposition on <i>Phyllostachys edulis</i> (Carr.) seedlings under different watering conditions: is seedling drought tolerance related to nitrogen metabolism? Plant and Soil. 448(1-2): 539-552. Retrieved from ://WOS:000516451200004. doi: 10.1007/ s11104-020-04445-z.

Song, S., Li, X.M., Wang, X., Zhou, Q., Li, Y.P., Wang, X.J. and Dong, S.K. (2022). Effects of drought stress on key enzymes of carbon metabolism, photosynthetic characteristics and agronomic traits of soybean at the flowering stage under different soil substrates. Phyton-International Journal of Experimental Botany. 91(11): 2475-2490. Retrieved from //WOS:000829621100007. doi: 10.32604/ phyton.2022.021090.

Vezina, L.P. and Langlois, J.R. (1989). Tissue and cellular distribution of glutamine synthetase in roots of pea (*Pisum sativum*) seedlings. Plant Physiology. 90(3): 1129-1133. Retrieved from //MEDLINE:16666862. doi: 10.1104/pp.90.3.1129.

Wang, L., Liu, L., Pei, Y., Dong, S., Sun, C., Zu, W. and Ruan, Y. (2012). Drought resistance identification of soybean germplasm resources at bud stage. Journal of Northeast Agricultural University. 43(1): 36-43. Retrieved from // CSCD:4447907.

Wang, X., Tian, Y.M., Liu, S.B., Wang, X.Y., Wu, Z.H. and Dong, S.K. (2023). Dynamic response of nitrogen content to moisture variation in soybean during the whole growth period. Legume Research. 46(4): 489-495. Retrieved from //WOS:000988143700015. doi: 10. 18805/lrf-723.

Xing, X.H., Xui, Z.J., Qi, Y.J., Wang, X.J., Sun, D.L., Bian, N.F. and Wang, X. (2018). Effect of exogenous alpha-naphthaleneacetic acid on carbon metabolism of soybean under drought stress at flowering stage. Ying yong sheng tai xue bao =The journal of applied ecology. 29(4): 1215-1224. Retrieved from //MEDLINE:29726231. doi: 10.13287/j.1001-9332.201804.022.

Zhou, Q., Song, S., Wang, X., Yan, C., Ma, C.M. and Dong, S.K. (2022). Effects of drought stress on flowering soybean physiology under different soil conditions. Plant Soil and Environment. 68(10): 487-498. Retrieved from < //WOS: 000874683700003. doi: 10.17221/237/2022-pse64200109. doi: 10.1038/s41598-022-09611-0.