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RESEARCH ARTICLE

Effects of drought stress and re-watering on nitrogen content in soybean at different growth stages

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ABSTRACT

Drought stress is a major limiting factor for soybean (*Glycine max* (L.) Merr.) growth and yield in arid and semi-arid regions. This study investigates the N content in different organs and the yield impact of two drought-tolerant soybean cultivars, Hei Nong44 and Hei Nong65, under varying drought intensities and re-watering at different growth stages. The results indicate that the effect of drought stress on N content varies with growth stage and organ. During the seedling and flowering stages, the ammoniacal N content in leaves and petioles of both cultivars generally decreased, with a reduction of 16.04%-21.95% during seedling stage and 6.46%-23.52% during flowering stage under severe drought (S), while it increased in stems. The nitrate N content in leaves peaked under moderate drought during the seedling stage and under severe drought during the flowering stage, increasing by 20.00% and 16.21%, 15.71% and 55.56% for 'Hei Nong44' and 'Hei Nong65', respectively, while it decreased in stems. During the pod-filling stage, ammoniacal N content increased in leaves and stems, with an increase of 20.99%-60.13% under S, while it showed a rising-then-falling trend in pods. Nitrate N content had the highest increase in leaves and stems, rising by 67.02%-69.31% under S, while it decreased in petioles with increasing drought severity. After re-watering, both cultivars showed some recovery in N content. Yield reduction was more significant during the flowering and pod-filling stages, decreasing by 17.45%-32.66% and 21.47%-35.63%, respectively.

Key words: Compensation effect, *Glycine max*, growth stage, nitrogen levels, water stress.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most widely cultivated crops in the legume family, which can provide not only plant oil and protein for human needs, but also various bioactive substances beneficial to human health, such as soybean isoflavones, lecithin, peptides, dietary fiber, etc. (Hao et al., 2013; Basal et al., 2020). China, as one of the largest soybean consumers in the world, is estimated to have a soybean demand of about 133 million tons by 2035, which will bring huge pressure on domestic soybean production and import (Feng, 2021; Chandio et al., 2022). Therefore, increasing domestic soybean yield will be a key measure to alleviate this pressure.

The growth, development and yield of soybean are affected by various abiotic stress factors, such as drought, flooding, extreme temperature, cold, mineral toxicity, etc. (Rane et al., 2021), and drought, as one of the most limiting variables among abiotic stress factors, seriously affects quality and yield of soybean. Moreover, due to the global climate change, which alters the amount and timing of precipitation, drought may become more frequent under the projected climate scenarios, especially in tropical and subtropical regions (Haile et al., 2020). Soybean is a drought-sensitive crop, and its yield response to drought stress varies depending on the growth stage. Previous studies have shown that, compared with adequate water supply, drought at any growth stage will reduce yield; however, the most significant yield loss occurred when drought happened at flowering-pod setting stage (Wei et al., 2018). In natural environment, drought and re-watering always occur alternately, especially under climate change conditions, this change will become more frequent (Qi et al., 2021). The change of water may directly limit plant growth and development, photosynthesis and many metabolic pathways (Kaur et al., 2021). The compensatory effect of rewatering on plant growth after drought has also been confirmed by many experimental studies (Li et al., 2019; 2021). However, whether re-watering can fully recover after drought stress may depend on the drought intensity or duration before re-watering.

Nitrogen is an essential basic nutrient element for all life, and also the nutrient element with the largest demand for crops. Its content is crucial for plant growth structure, nucleotide and enzyme synthesis, and many other biochemical reactions (Kaur et al., 2017). Although the atmosphere contains 59%-78% N gas, plants cannot use it directly and need to convert it into usable combined N. Nitrogen can easily be transformed between various reduced and oxidized states, and migrate and cycle through hydrological and atmospheric processes (Hofman and van Cleemput, 2004). Plants exhibit various adaptive changes to optimize the acquisition of N in various forms, which increase the complexity of plant N nutrition. Therefore, plants can use various chemical forms of N, from simple inorganic N compounds (such as ammoniacal N and nitrate N) to organic N forms (such as amino acids) (Ohyama, 2010; Farzadfar et al., 2021). However, most related studies have focused on the physiological responses of crop N metabolism to single drought levels or different levels of drought and re-watering at a single period. There is a lack of exploration into the changes in N content in crops under different drought stresses at various periods and re-watering after drought, as well as the impact of re-watering after drought at different periods on the final yield. Therefore, this study selected two soybean cultivars, the drought-tolerant ‘Hei Nong44’ and the drought-sensitive ‘Hei Nong65’, as experimental materials. The aim was to systematically investigate the changes in ammoniacal N and nitrate N content in different organs of soybeans under different drought stresses and re-watering at various time periods, and to determine the impact on the final yield of soybeans. And also to elucidate the mechanisms by which drought and rehydration impact the N content and yield of soybeans, and to provide a theoretical foundation for enhancing the drought resistance of soybeans and improving agricultural production efficiency.

MATERIALS AND METHODS

Experimental materials and methods.

The experiment was conducted at the experimental practice base of Northeast Agricultural University in Harbin, China, using two soybean cultivars (*Glycine max* (L.) Merr.) The cultivars were ‘Hei Nong44’ (drought-tolerant) and ‘Hei Nong65’ (drought-sensitive) (Wang et al., 2012). The experiment commenced in late spring (May) of 2021 and continued until early autumn (September). The temperature variations are shown in Figure 1. The monthly average air humidity from May to September was 51%, 65%, 77%, 78%, and 70%, respectively. The average daily photoperiod from May to September was 14.93, 15.64, 15.28, 14.07, and 12.53 h, respectively. The experiment utilized clay loam soil (international classification), with a pH of 6.70, cation exchange capacity of 238.60 mmol kg^{-1}, an organic C content of 23.52 g kg^{-1}, and total N, P, and K contents of 1.42, 0.62, and 25.53 g kg^{-1}, respectively. The nitrate N content was 46.26 mg kg^{-1} and the available P content was 11.16 mg kg^{-1}. No fertilizers were applied throughout the entire growth period.

The specific experimental method was as follows: A pot experiment was conducted in a glass rain shelter at the experimental training base of Northeast Agricultural University. The primary function of the shelter was to block rainwater, allowing better control of moisture conditions while permitting natural light to enter. This setup prevents rainfall interference and maintains temperature and humidity consistent with outdoor conditions, thereby better simulating the natural growth environment of soybeans. Plastic pots with a diameter of 26 cm and a height of 33 cm were used, each filled with 12 kg air-dried soil. Healthy, pest-free soybean seeds were selected for sowing, with three seedlings retained per pot. Water content was controlled using a combination of the gravimetric method and a soil moisture sensor (ECH$_2$O-TE/EC-TM, EM-50, Decagon Devices, Pullman, Washington, USA). Drought stress was categorized into four levels: Mild stress (soil moisture content at 50%-60% field capacity, denoted as L), moderate stress (soil moisture content at 40%-50% field capacity, denoted as M), severe stress (soil moisture content at 30%-40% field capacity, denoted as S), and normal irrigation (soil moisture content at 65%-75% field capacity). Due to slight variations in the time required to reach the predetermined drought levels, control samples were taken simultaneously with each drought treatment. These controls were denoted as CK-L, CK-M, and CK-S, corresponding to the respective drought levels.

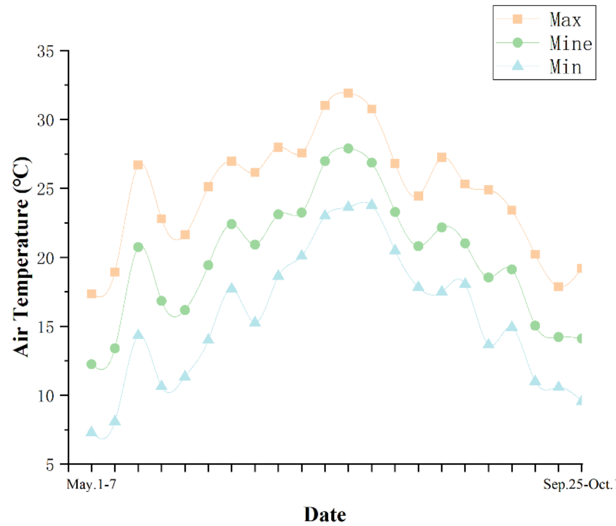

Figure 1. Weekly average maximum, minimum, and mean temperatures in Harbin, Heilongjiang Province, China, from 1st May to 1st October 2021. Meteorological data were measured by the Northeast Agricultural University weather station.

A completely randomized design was employed involving two cultivars (‘Hei Nong44’ and ‘Hei Nong65’) and three growth stages: Seedling stage, flowering stage, and pot filling stage, corresponding to V3, R2, and R5 stages respectively. The experiment included six treatments under four water conditions (L, M, S, and their drought controls CK-L, CK-M, CK-S), along with corresponding drought re-watering treatments (RL, RM, RS, RCK), resulting in a total of 252 pots. These were divided into 90 control pots and 162 treatment pots. The latter were further subdivided into drought, re-watering, and yield measurement groups, each consisting of 54 pots. Drought stress during the seedling stage began at the V3 stage. The control group received normal watering throughout the entire growth period. The treatment group was fully watered until reaching the designated stage, after which watering was stopped to induce natural drought conditions. Soil moisture content in the treatment group was measured daily. Upon reaching the predefined mild stress range, three pots with uniform growth were randomly selected from both the control and treatment groups between 08:00-09:00 h for sampling. Simultaneously, three pots of plants under the same stress level were randomly selected for re-watering to restore soil moisture to control levels, followed by full watering until the R2 stage before sampling. The same process applied to moderate and severe stress levels. Sampling times for the control and treatment groups were consistent. Samples were stored in a refrigerator immediately after collection and transported to the laboratory. Each treatment was replicated three times. Drought stress during the flowering and grain-filling stages was induced at the R2 and R5 stages respectively, with re-watering and sampling occurring at the R4 and R6 stages. After completing drought and re-watering treatments at different stages (V3, R2, R5), three pots from each treatment (RL, RM, RS, RCK) of both cultivars were maintained under normal watering for yield measurement.

Determination of ammoniacal N content

The ninhydrin colorimetric method was used. Accurately weigh 0.05 g sample and put it into a 50 mL triangular flask. Add 5 mL 3% acetic acid and soak for 5 h. Add 25 mL distilled water and shake for 30 min. Filter and take 0.5 mL filtrate in a stoppered test tube. Shake well and cover with a spherical cap. Put it in boiling water and heat for 15 min. Take it out and stir and cool for 15 min. Color was measured at 580 nm.

Determination of N content

A plant sample (0.5 g) was added into a 50 mL Erlenmeyer flask, 25 mL distilled water, 0.1 g calcium sulfate, and a measured quantity of activated C were added. The mixture was agitated for 30 min and then filtered; 10

mL filtrate were put in an 8 cm diameter evaporating dish. Calcium carbonate (0.05 g) was introduced and the mixture was evaporated to dryness using a water bath. Upon cooling, 2 mL phenoldisulfonic acid were added and allow react for 10 min. Following this, 10 mL water were added and stirred until the residue dissolved completely. After the solution was cooled, 7 mL ammonia water were incrementally added until a yellow hue developed, then additional 3 mL ammonia water were incorporated. The resultant solution was transferred to a 50 mL volumetric flask and make up to the mark. The solution was spectrophotometrically analyzed at 410 nm.

Determination of yield

Employing an electronic balance, the three pots of plants preserved post-treatment were subjected to manual threshing to assess the yield. Concurrently, the weight of 100-seed weight and the ultimate yield for each treatment were ascertained.

Analysis software

Microsoft Office Excel 2010 was used to draw and process all the relevant data. Data were subjected to statistical scrutiny utilizing IBM SPSS software (Version 21.0: IBM Corporation, Armonk, New York, USA). Duncan's one-way test was applied to ascertain significance at a 5% threshold. Origin 9 (OriginLab Corporation, Northampton, Massachusetts, USA) was used to create the statistical graphs.

RESULTS AND DISCUSSION

Effects of drought and re-watering on N content at seedling stage

As shown in Figure 2(A), drought stress caused the ammoniacal N content of leaves and petioles of both cultivars to decrease, and reached the lowest level under severe drought, 'Hei Nong44' and 'Hei Nong65' were 19.19% and 16.04%, 19.53% and 21.95% lower than CK-S, respectively. However, the ammoniacal N content of stems showed an increasing trend, and the increase was the largest under severe drought, 'Hei Nong44' and 'Hei Nong65' were 33.53% and 33.76% higher than CK-S, respectively. As shown in Figure 2B, after re-watering, the ammoniacal N content of both cultivars recovered to different extents. Among them, the compensation effect of petioles under mild stress was the most obvious, 'Hei Nong44' and 'Hei Nong65' increased by 28.47% and 36.50%, respectively; the stem of both cultivars was still higher than RCK after re-watering, but the change range decreased.

As shown in Figure 2C, under mild and moderate drought stress, the nitrate N content of leaves of both cultivars increased compared with CK-L and CK-M, while it decreased under severe drought, 'Hei Nong44' and 'Hei Nong65' were 20.00% and 15.71% higher than CK-M, respectively. In the stems, the variation in nitrate N content between the two cultivars was not significant. However, in the petioles, 'Hei Nong44' exhibited a significant decrease, showing a reduction of 3.27% under severe drought conditions compared to CK-S. In contrast, 'Hei Nong65' did not show a significant change. The nitrate N content of stems and petioles did not change significantly under different degrees of drought stress. As shown in Figure 2D, after re-watering, the nitrate N content of both cultivars recovered to some extent, among which the nitrate N content of leaves and petioles of 'Hei Nong44' recovered to the CK level, while the nitrate N content of stems increased the most significantly after re-watering under severe drought, increased by 4.63%. However, the performance of 'Hei Nong65' was slightly different from that of 'Hei Nong44'. After re-watering under stress, except for the recovery to RCK in petioles, the nitrate N content of leaves and stems did not recover to the control level after severe drought, and their contents decreased by 8.14% and 12.22%, respectively.

The seedling stage is crucial for plant nutrition growth, and drought stress can impair root growth and reduce water and nutrient uptake by plants. Ammoniacal N is essential for the synthesis of amino acids and proteins, which are vital for plant growth and development. Previous studies have shown that amino acids are the main form and transport form of N compounds in plants, whether they are newly assimilated or redistributed. Amino acids not only connect N absorption, assimilation and protein synthesis and degradation in organs, but also enable N distribution, transfer and redistribution between source and sink (Yang et al., 2020; Aluko et al., 2023). We observed that drought stress decreased the ammoniacal N content of leaves and petioles, especially in leaves, while it increased the ammoniacal N content of stems. This could be explained by the fact that plants under drought conditions use more ammoniacal N to produce energy and assimilates, and to synthesize other compounds that help them cope with stress. Plants also regulate the distribution and transport of N, and move N and other nutrients from leaves to stems or other parts, resulting in lower ammoniacal N content in leaves and petioles, and higher ammoniacal N content in stems, sustaining the normal growth of the whole plant. Nitrate N is an important form of inorganic N, which plays a role in plant growth, osmotic regulation and abiotic stress adaptation (Zhang et al., 2018; Ye et al., 2022). We found that the nitrate N content of leaves increased significantly under mild and moderate stress, but decreased under severe stress; the changes in stems and petioles were nonsignificant. Some studies have reported that drought stress will decrease the nitrate N content in roots, while increasing the nitrate N content in leaves, and reduce the nitrate reductase (NR) activity in leaves, which may be associated with the reduction of photosynthesis rate due to stomatal closure (Xia et al., 2020). Some researchers have also noted that different varieties of the same plant have different drought resistance and recovery abilities in the process of drought and re-watering (Wang et al., 2022a), and plants will show a compensation effect after re-watering. We found that the recovery ability of ‘Hei Nong65’ was much lower than that of ‘Hei Nong44’, which may be because the sensitive variety had more severe changes in ammoniacal N content under drought conditions, and thus required more time to resume its normal growth after re-watering. The differences between different drought-resistant varieties will affect their recovery abilities after re-watering, and the recovery speed of drought-resistant varieties is faster than that of sensitive varieties.

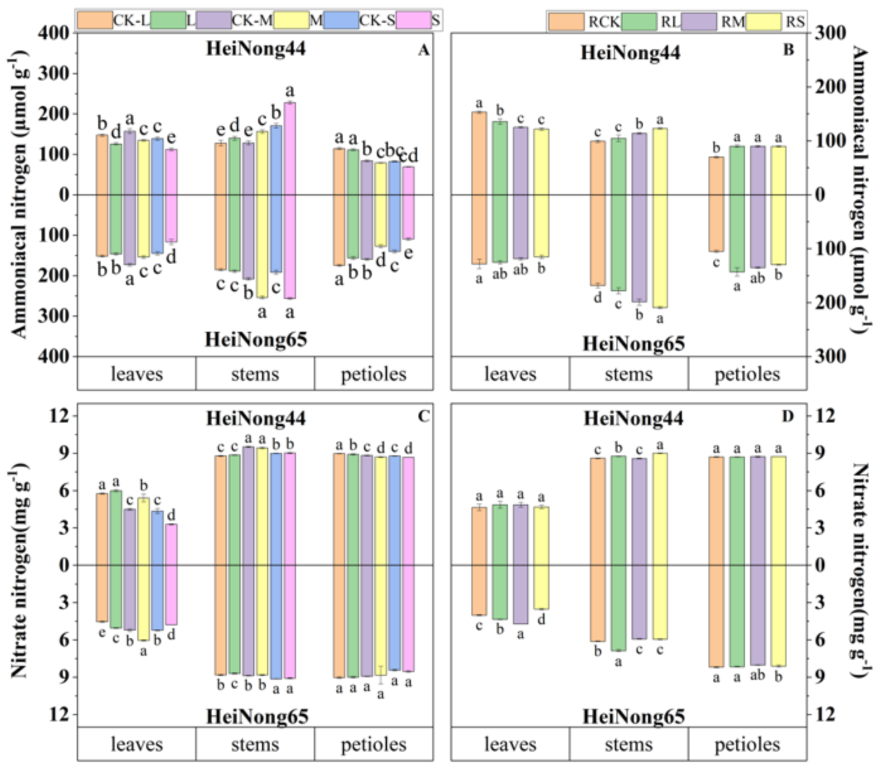

Figure 2. Nitrogen content of ‘Hei Nong44’ and ‘Hei Nong65’ soybeans under drought and re-watering at seedling stage. Values are expressed as the mean ± SD of three replicates. Different letters indicate significant differences in N content within the same organ under different drought and re-watering conditions ($P < 0.05$). A: Ammoniacal N content in different plant parts under varying drought conditions; B: Ammoniacal N content in different plant parts after re-watering following different drought levels; C: Nitrate N content in different plant parts under varying drought conditions; D: Nitrate N content in different plant parts after re-watering following different drought levels. L: Mild drought; M: moderate drought; S: severe drought; CK-L; CK-M; CK-S: control groups corresponding to mild; moderate; and severe drought conditions; respectively. RCK: normal water supply; RL: re-watering after mild drought; RM: re-watering after moderate drought; RS: re-watering after severe drought.

Effects of drought and re-watering on N content at flowering stage

As shown in Figure 3A, the changes of ammoniacal N content in two cultivars under drought stress at flowering stage were similar to those at seedling stage. In 'Hei Nong44', the leaf ammoniacal N content decreased by 22.17% under severe drought stress compared to CK-S. Although the ammoniacal N content in the petioles decreased under moderate and severe drought conditions, the changes were nonsignificant. In contrast, in 'Hei Nong65' the ammoniacal N content in both leaves and petioles gradually decreased with increasing drought stress, reaching the lowest levels under severe drought conditions, with reductions of 23.52% and 19.84% compared to CK-S, respectively. However, the ammoniacal N content in stems showed an increasing trend, especially under severe drought, which increased by 50.25% and 48.07% for 'Hei Nong44' and 'Hei Nong65', respectively, compared with CK-S. As shown in Figure 3B, after re-watering at flowering stage, the ammoniacal N content in leaves of both cultivars was still lower than that in CK, but the loss was alleviated. The stems showed a more obvious compensation effect after re-watering, which increased by 16.52% and 17.01% for 'Hei Nong44' and 'Hei Nong65', respectively, under severe stress after re-watering. Among them, 'Hei Nong44' recovered to the RCK level in all treatments, and 'Hei Nong65' also recovered to the RCK level except for severe drought, while the changes in petioles were nonsignificant.

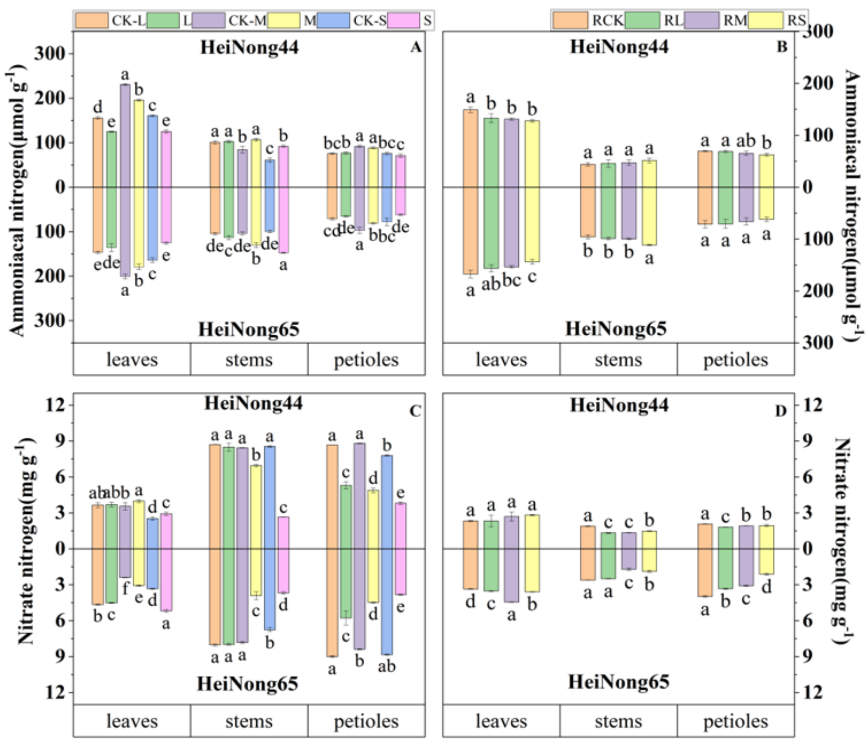

Figure 3. Nitrogen content of 'Hei Nong44' and 'Hei Nong65' soybeans under drought and re-watering at flowering stage. Values are expressed as the mean ± SD of three replicates. Different letters indicate significant differences in N content within the same organ under different drought and re-watering conditions ($P < 0.05$). A: Ammoniacal N content in different plant parts under varying drought conditions; B: Ammoniacal N content in different plant parts after re-watering following different drought levels; C: Nitrate N content in different plant parts under varying drought conditions; D: Nitrate N content in different plant parts after re-watering following different drought levels. L: Mild drought; M: moderate drought; S: severe drought; CK-L; CK-M; CK-S: control groups corresponding to mild; moderate; and severe drought conditions; respectively. RCK: normal water supply; RL: re-watering after mild drought; RM: re-watering after moderate drought; RS: re-watering after severe drought.

As shown in Figure 3C, the changes of nitrate N content in different parts under drought stress at flowering stage were different. The nitrate N content in leaves of both cultivars did not change significantly under mild drought, but increased significantly under moderate and severe drought, which increased by 16.21% and 55.56% for 'Hei Nong44' and 'Hei Nong65', respectively, compared with CK-S under severe drought. In contrast, the nitrate N content in stems and petioles decreased continuously with the aggravation of drought, and the decreasing trend in petioles was the most obvious. Under severe drought, the nitrate N content in stems and petioles of 'Hei Nong44' and 'Hei Nong65' decreased by 68.85% and 51.08%, 41.19% and 56.65%, respectively, compared with CK-S. As shown in Figure 3D, the nitrate N content in all parts recovered to different extents after re-watering at flowering stage, but only the leaves could recover to the normal level, while the stems and petioles were still lower than the RCK level, which decreased by 21.74% and 6.38%, 27.89% and 46.99% for 'Hei Nong44' and 'Hei Nong65', respectively.

The flowering stage is a critical period in the transition of plants from vegetative growth to reproductive growth. Compared to the vegetative stage, the water requirement for maintaining normal growth in soybeans during this time (R1 and R2 stages) doubles, needing an increase in weekly rainfall from 0.7 inches to 1.4 inches., and the nutrients were preferentially allocated to reproductive organs to promote development and reproduction (Zhou et al., 2019; Matcham and Conley, 2020). Some studies have shown that at R2 stage, the roots transport the absorbed N to the leaves, and then distribute it to the new leaves and flowers (Tegeder and Masclaux-Daubresse, 2018). Our study found that although the changes of ammoniacal N content in various organs under drought at flowering stage were similar to those at seedling stage, the content in leaves and petioles decreased more significantly, which also indirectly reflected the water demand at flowering stage. Drought would reduce the vitality of pollen, affect the normal pollination and seed setting, and thus affect the yield (Alqudah et al., 2011; Guo et al., 2023). We also found that under drought at flowering stage, the ammoniacal N and nitrate N content in stems and petioles were lower than that in leaves, which might be because stems and petioles acted as the main transport channels of N, and transferred more N to leaves and flowers, to maintain the photosynthetic ability and reproductive process of leaves, and thus maintain their yield. In addition, some studies have suggested that part of the nitrate N absorbed by roots is transported to leaves along the xylem and participates in the synthesis of amino acids, and the remaining part is stored in roots, stems and other parts, to promote the timely supply of N (Mahboob et al., 2023). Our study also found this phenomenon, at seedling and flowering stage, the nitrate N content in stems and petioles was higher than that in leaves, but it was more affected by drought at flowering stage, and the higher the drought degree, the greater the decrease of its content. While the nitrate N content in leaves increased, which might be the physiological adaptation response of plants, by redistributing the nitrate N from stems, petioles and other parts to leaves, to maintain the photosynthetic activity and intracellular water. When re-watering after drought at flowering stage, the changes of ammoniacal N and nitrate N were similar to those at seedling stage, but the ammoniacal N and nitrate N content in stems and petioles decreased significantly, which might be because soybean entered the reproductive growth stage, and N was transferred to pods, and stems and petioles mainly acted as transport roles. At the same time, our study found that even after re-watering, the nitrate N content in stems and petioles was still lower than that under normal water conditions, indicating that drought caused irreversible damage to them.

Effects of drought and re-watering on N content at pod-filling stage

As shown in Figure 4(A), under different drought levels, the ammoniacal N content in both the leaves and stems of the two soybean cultivars increased. The leaf ammoniacal N content of 'Hei Nong44' and 'Hei Nong65' rose by 20.99% and 46.14%, respectively, compared with CK-M under moderate drought, but dropped under severe drought, though still significantly higher than CK-S. The stem ammoniacal N content reached the highest under severe drought, and 'Hei Nong44' and 'Hei Nong65' increased by 45.76% and 60.13%, respectively, compared with CK-S. During the pod-filling phase, petiole ammoniacal N levels remained stable despite drought conditions. Conversely, the ammoniacal N in the pods initially rose, then fell, reaching its highest level during moderate drought stress. Specifically, 'Hei Nong44' and 'Hei Nong65' showed increases of 25.21% and 9.49%, respectively, when compared to the CK-M. The pods had much higher ammoniacal N content than leaves, stems and petioles. As shown in Figure 4B, after re-watering, the ammoniacal N content of leaves, stems and petioles of both cultivars showed some recovery effect. The recovery effect of 'Hei Nong44' and 'Hei Nong65' in leaves and stems was more noticeable, and they increased by 15.25%, 18.77% and 15.44%, 38.35%, respectively, after re-watering under severe stress, suggesting that 'Hei Nong65' had a stronger recovery effect than 'Hei Nong44'.

Meanwhile, the ammoniacal N content of pods of both varieties returned to the RCK level after re-watering under mild drought, but failed to recover after re-watering under moderate and severe drought, indicating that pods were more affected by drought at the pod-filling stage, and the re-watering duration was insufficient to fully restore their function.

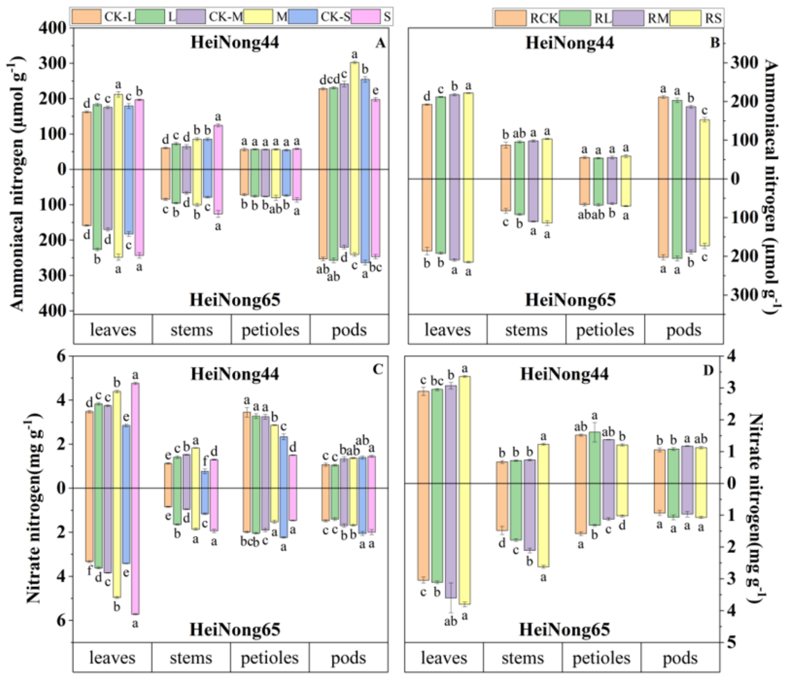

Figure 4. Nitrogen content of ‘Hei Nong44’ and ‘Hei Nong65’ soybeans under drought and re-watering at pod-filling stage. Values are expressed as the mean ± SD of three replicates. Different letters indicate significant differences in N content within the same organ under different drought and re-watering conditions ($P < 0.05$). A: Ammoniacal N content in different plant parts under varying drought conditions; B: Ammoniacal N content in different plant parts after re-watering following different drought levels; C: Nitrate N content in different plant parts under varying drought conditions; D: Nitrate N content in different plant parts after re-watering following different drought levels. L: Mild drought; M: moderate drought; S: severe drought; CK-L; CK-M; CK-S: control groups corresponding to mild; moderate; and severe drought conditions; respectively. RCK: normal water supply; RL: re-watering after mild drought; RM: re-watering after moderate drought; RS: re-watering after severe drought.

As shown in Figure 4C, illustrates a marked decrease in the nitrate N levels across different plant tissues upon reaching the pod-filling stage, with stems and petioles showing the most significant reduction. Drought conditions led to an elevation in the nitrate N levels in leaves and stems, which was particularly pronounced during intense drought. Under such conditions, ‘Hei Nong44’ and ‘Hei Nong65’ experienced increases in nitrate N levels in leaves and stems by 67.02% and 69.31%, and 67.25% and 66.42%, respectively, compared with CK-S. On the other hand, the nitrate N content in petioles inversely correlated with the severity of drought, decreasing by 35.69% and 34.50% for ‘Hei Nong44’ and ‘Hei Nong65’, respectively, under severe drought stress. The nitrate N content in the pods did not show significant changes under any of the treatments. As shown in Figure 4D, after re-watering under stress at the pod-filling stage, the nitrate N content of leaves of both cultivars showed a significant recovery effect, especially ‘Hei Nong44’ had a faster recovery ability after re-watering under moderate and severe drought, and ‘Hei Nong44’ and ‘Hei Nong65’ increased by 16.26% and 25.00%, respectively. Under mild and moderate drought stress, the nitrate N content in the stems and petioles of ‘Hei Nong44’ showed varying degrees of recovery after re-watering. However, after severe stress, the nitrate N content in the stems and petioles did not recover to RCK levels. In contrast, for 'Hei Nong65', even after re-watering following mild stress, the nitrate N content in the stems and petioles struggled to return to RCK levels. Overall, the nitrate N content in the pods of both cultivars showed a slight increase after re-watering, but this increase was nonsignificant.

The N content of soybean varied after entering the pod-filling stage, which might be related to its physiological and metabolic needs and responses to drought. At the flowering stage, the plant entered the reproductive growth stage, and the demand for N increased, mainly to support the development and formation of flowers and seeds (Yun et al., 2023). However, as the plant entered the pod-filling stage, the ammoniacal N and nitrate N content of leaves, stems and petioles started to decrease, while the ammoniacal N content of pods was much higher than that of other organs, indicating that N was transferred to the pods for seed development. Du et al. (2020) observed that as the soybean growth process continued, the N content in the leaves gradually decreased, suggesting that N was transferred from the leaves to the seeds to meet the needs of seed development and growth; meanwhile, drought stress significantly increased the amino acid and nitrate content in the leaves, especially during R5-R6. We found that the ammoniacal N and nitrate N content of leaves and stems increased to different extents under drought at the pod-filling stage, which might be because nitrate N was not only an important nutrient for cells, but also an osmotic protector for cell membrane damage (Krouk et al., 2010). The ammoniacal N content at the pod-filling stage was different from that at the seedling and flowering stages, which might be because the leaves were active in growth at the seedling and flowering stages, and the demand for N was high, while at the pod-filling stage, the N stored in the leaves gradually transferred to the pods, and drought hindered this transfer, because the water content in the plant decreased, affecting the transport of nutrients, resulting in the accumulation of ammoniacal N content in the leaves and stems. After re-watering, the N content of various organs recovered, but the study found that even after timely re-watering after stress, the N level in the leaves and pods still could not be recovered under moderate and severe stress. It was also found that 'Hei Nong44' had better recovery ability than 'Hei Nong65' in various organs after re-watering under mild and moderate stress, and overall, even the drought-tolerant varieties could not fully recover the impact of drought after re-watering under severe drought.

Comparison of N between the two cultivars at different growth stages

As shown in Figures 5A and 5C, the ammoniacal and nitrate N contents of different organs of the two cultivars changed to different extents under different drought conditions compared with the control. Among them, the changes of ammoniacal and nitrate N contents were most obvious under severe drought condition. The drought stress at different growth stages caused the changes of ammoniacal N content mainly in the stem and leaf, while the changes of nitrate N content mainly concentrated in the flowering and pod-filling stages. As shown in Figures 5B and 5D, after re-watering, the change trends of the two cultivars in different organs were slightly different, but generally showed similar to the drought treatment. Overall, after severe drought and re-watering, the ammoniacal and nitrate contents of the two cultivars both recovered significantly. This might be because after re-watering, the plants could quickly absorb the N in the soil to compensate for the loss during drought, thus making the N content rise rapidly. To further analyze this change, we compared the ammoniacal and nitrate contents of the two cultivars under severe drought and re-watering with those of the control group.

As shown in Figure 6, the ammoniacal and nitrate contents of different organs of the two soybean varieties were close to 1, that is, CK, after severe drought and re-watering, suggesting that the N level could be quickly restored after re-watering. In general, the two cultivars recovered well to the normal level after severe drought and re-watering at the seedling stage, but the N contents of the two cultivars differed after severe drought and re-watering at the flowering stage. The ammoniacal contents of the two varieties returned to the level similar to the control, while the nitrate contents showed some recovery, but did not reach the normal level. At the pod-filling stage, the N contents of the two cultivars after re-watering exhibited some variations. The ammoniacal contents after severe drought and re-watering were higher in the leaf, stem and petiole, but lower in the pod. Only 'Hei Nong44' had some compensation effect, while 'Hei Nong65' had no apparent compensation effect. Meanwhile, the nitrate content returned to the level similar to CK in the petiole and pod after re-watering, but was lower than CK in the leaf and stem. This indicates that the compensation effect of re-watering after drought stress depends on the drought degree, stage and organ. Severe drought would diminish the compensation effect, while drought-tolerant varieties have stronger N metabolism and compensation effect.

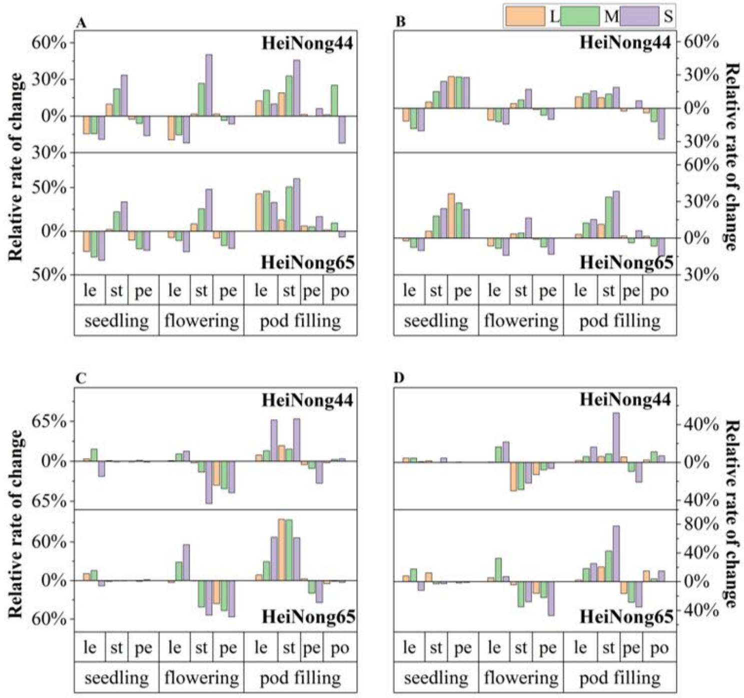

Figure 5. Change rate of N content of 'Hei Nong44' and 'Hei Nong65' soybeans under drought and re-watering in each period. A: Ammoniacal N content under drought stress; B: Ammoniacal N content after re-watering corresponding to drought stress; C: Nitrate N content under drought stress, D: Nitrate N content after re-watering corresponding to drought stress. le: Leaf; st: stem; pe: petiole; po: pod; L: mild drought; M: moderate drought; S: severe drought.

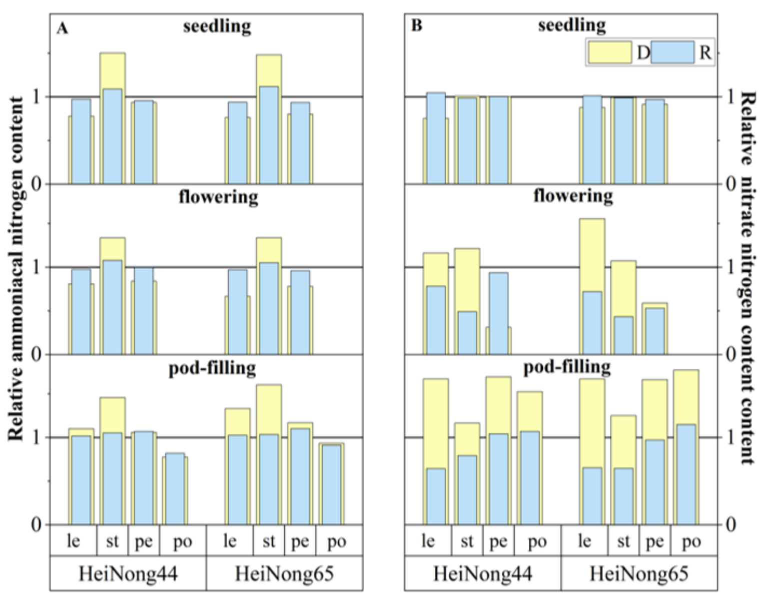

Figure 6. Relative N content of 'Hei Nong44' and 'Hei Nong65' soybeans under severe drought and re-watering in various periods. A: Severe drought and re-watering ammoniacal N content changes; B: severe drought and re-watering nitrate N content changes. le: Leaf; st: stem; pe: petiole; po: pod; D: drought; R: re-watering.

Effect of drought and re-watering on yield

As shown in Figures 7A and 6B, the yields of two soybean cultivars exhibit a decreasing trend with increasing drought stress at different growth stages. Under RS conditions, the impact on yield at all growth stages is most significant. Compared to RCK, the yields of 'Hei Nong44' and 'Hei Nong65' decreased by 21.67%, 27.67%, and 30.50%, and 19.45%, 32.66%, and 35.63% during the seedling, flowering, and pod filling stages, respectively. In contrast, under RL conditions, the yield reductions were relatively smaller. Compared to RCK, the yields of 'Hei Nong44' and 'Hei Nong65' decreased by 4.27%, 17.45%, and 21.47%, and 9.55%, 23.02%, and 22.46% during the seedling, flowering, and pod filling stages, respectively. Additionally, we found that the yield reductions under RM conditions during the flowering stage and RL conditions during the pod-filling stage were comparable to those under RS conditions during the seedling stage, indicating that drought stress during the flowering and pod-filling stages has a more significant impact on soybean yield.

As shown in Figures 7C and 7D, the 100-seed weight of both soybean cultivars decreases to varying degrees with increasing drought stress, a trend similar to that observed for yield. Under RS conditions, the reductions in hundred-seed weight were more pronounced during the flowering and pod-filling stages. Compared to RCK, the hundred-seed weights of 'Hei Nong44' and 'Hei Nong65' decreased by 9.00% and 13.79%, and 14.33% and 17.14%, respectively. In contrast, the impact of drought stress during the seedling stage on hundred-seed weight was relatively minor. Under RS conditions, compared to RCK, the hundred-seed weights of 'Hei Nong44' and 'Hei Nong65' decreased by only 4.99% and 5.23%, respectively.

Drought stress significantly affects plant root systems, photosynthesis, and the synthesis and transport of nutrients, ultimately impacting plant growth, development, and final yield (Seleiman et al., 2021; Zia et al., 2021; Ding et al., 2022). Studies have shown that drought stress during the flowering and pod-setting stages has a more pronounced effect on soybean yield compared to the seedling stage, with yield reductions ranging from 24% to 82% during the flowering and pod-setting stages, compared to 10% to 33% during the seedling stage (Wei et al., 2018). Our research also found that the impact of drought stress on soybean yield varies across different growth stages. During the seedling stage, different levels of drought stress resulted in yield reductions of 4.27% to 21.67% for two soybean varieties. In contrast, during the flowering and grain-filling stages, yield reductions were 17.45% to 32.66% and 21.47% to 35.63%, respectively. These findings are consistent with those of Jumrani and Bhatia (2018), who reported that drought stress during the V4 (vegetative) and R5 (reproductive) stages led to seed yield reductions of 28% and 74%, respectively, compared to the control group without drought stress. This indicates that drought stress during the R5 stage has a much greater impact than during the V4 stage. The significant yield reduction during the flowering and grain-filling stages can be attributed to the increased water demand of soybean plants during these stages. Additionally, some researchers believe that soybean plants experiencing drought during the seedling stage, due to their smaller leaf area, lower photosynthetic rate, and smaller biomass, may partially recover after rehydration. In contrast, drought stress during the flowering and grain-filling stages occurs later, leading to higher flower drop rates and reduced numbers of developing pods and seeds, leaving less opportunity for recovery and resulting in significant yield losses (Jumrani et al., 2017; Wang et al., 2022b; Poudel et al., 2023). Research also indicates that the primary reasons for reduced seed yield are an increase in the number of empty pods, a decrease in the number of seeds per plant, and a reduction in the 100-seed weight (Basal and Szabó, 2020). Our study shows that under varying drought conditions followed by rehydration, 100-seed weight decreased to different extents. The two varieties showed reductions of 0.35% to 5.23% during the seedling stage, reductions of 3.90% to 14.33% during the flowering stage, and reductions of 12.07% to 17.14% during the grain-filling stage. Compared to yield, the reduction in 100-seed weight was relatively smaller, indicating that drought stress had a more significant impact on the number of seeds per plant rather than on seed size.

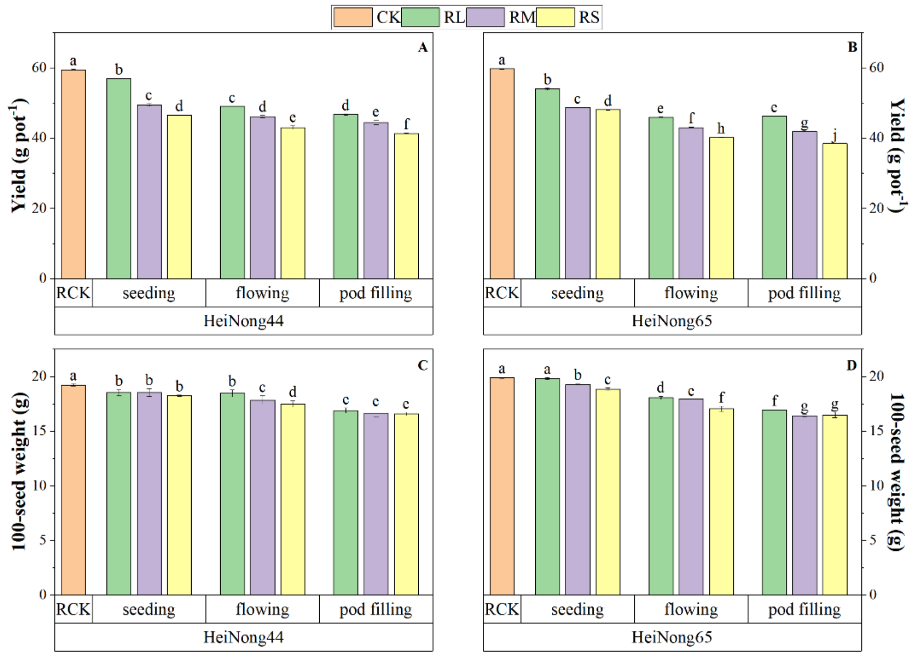

Figure 7. Effects of rewatering after drought at different stages on the yield and 100-grain weight of ‘Hei Nong44’ and ‘Hei Nong65’. Values are expressed as means of three replicates ± SD. Different letters indicate significant differences in N content within the same organ under different drought and rewatering conditions (P < 0.05). A: Yield of ‘Hei Nong44’ after rewatering following different levels of drought at different stages; B: Yield of ‘Hei Nong65’ after rewatering following different levels of drought at different stages; C: 100-Grain weight of ‘Hei Nong44’ after rewatering following different levels of drought at different stages; D: 100-Grain weight of ‘Hei Nong65’ after rewatering following different levels of drought at different stages. CK: Normal water supply; RL: rewatering after mild drought; RM: rewatering after moderate drought; RS: rewatering after severe drought.

CONCLUSIONS

These results indicate that there are differences in the N changes and recovery abilities of the two studied soybean cultivars in different growth stages and organs. At the seedling stage, the two cultivars could recover well to the normal ammoniacal and nitrate N contents; but at the flowering and pod-filling stages, although the ammoniacal content could recover well, the nitrate content was not fully recovered, and the recovery abilities of different organs showed differences, reflecting the complexity of the drought stress effects. In addition, compared with ‘Hei Nong65’, ‘Hei Nong44’ showed stronger compensation effect.

Author contribution

Conceptualization: S.D., X.Z. Methodology: S.D., X.Z. Software: X.Z., Z.Q. Formal analysis: S.D., X.Z., Z.Q., X.W. Investigation: S.D., X.Z. Resources: S.D. Data curation: S.D., X.Z., Z.Q., X.W. Writing-original draft: S.D. Writing-review & editing: S.D., X.Z., X.W. Visualization: X.Z., Z.Q. Supervision: S.D. Project administration: S.D. Funding acquisition: S.D. All co-authors reviewed the final version and approved the manuscript before submission.

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