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Effects of Irrigation with Sodium Nitroprusside on Physiological Characteristics of Soybean under Drought Stress

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ABSTRACT

Background: Although it is a key soybean-producing region, northeast China frequently faces drought. Drought severely affects soybean growth, development and even leads to yield reduction.

Methods: Sand cultivation method was employed to investigate the changes in malondialdehyde (MDA) content, nitric oxide (NO) content and individual leaf area of drought-tolerant soybean variety Heinong 44 (HN44) and drought-sensitive soybean variety Heinong 65 (HN65) under drought stress during the flowering stage, with different concentrations of sodium nitroprusside (SNP) used for irrigation.

Result: The NO and MDA contents of HN44 and HN65 leaves rose in response to drought stress, MDA content in HN44 and HN65 leaves was noticeably elevated, the individual leaf area showed a downward trend. Irrigation with SNP further increased NO accumulation and reduced membrane lipid peroxidation in the leaves, with the best effect observed at a concentration of 1000 μmol·g^{-1}. In practical production, SNP can be used to alleviate membrane lipid peroxidation damage in soybean leaves and improve soybean drought resistance when encountering drought.

Key words: Drought, Malondialdehyde, Sodium nitroprusside, Soybean.

INTRODUCTION

China is the birthplace of soybean [*Glycine max* (L.) Merr.], which is mostly farmed in the Yangtze River Delta, Jianghan Plain, Huang-Huai Plain and Northeast Plain. It is an important grain and cash crop, playing a significant role in agricultural production and people's livelihoods (Pagano and Miransari, 2016). Soybeans contain various nutrients such as proteins, fats, dietary fibers and isoflavones (Qin *et al.*, 2022; Tripathi and Misra, 2005). They are often processed into soy flour, soy milk, soy sauce and various soy-based products. The leftover soybean residue is also commonly used in the production of animal feed (Golbitz 1995; Sun *et al.*, 2021). The growth of soybeans is influenced by various factors, such as drought, pests and diseases and salinity-alkalinity. Drought is a natural phenomenon characterized by a prolonged lack of precipitation (Dai *et al.*, 2022), it has a major impact on agricultural output, environmental stability and local economic growth (Chen *et al.*, 2022). One of the essential processes in plant growth and development is the increase in leaf area, the leaf area of soybean plants is an important factor affecting yield. Studies by Peer *et al.* (2023); Lestari *et al.* (2019) have already indicated that drought stress can lead to a reduction in leaf area in plants. Therefore, it is highly necessary to enhance soybean's drought resistance capabilities.

When free radicalsattack the cell membrane lipid bilayer in plants, membrane lipid peroxidation occurs and MDA is produced (Ribeiro *et al.*, 2023). MDA, H_2O_2 and other chemical levels rise as a result of drought-stressed plants producing reactive oxygen species, or ROS (Begum *et al.*, 2023; Petrović *et al.*, 2023). When there is a significant accumulation of MDA within the plant body, lipid peroxidation is enhanced, which affects normal plant growth (Yasar *et al.*, 2008).

Being a very potent signaling molecule that controls the growth of plants, numerous developmental activities, such as stomatal movement, root formation and seed germination are influenced by NO (Lubyanova *et al.*, 2022; Pandey *et al.*, 2019). NO accumulates in plants when they face biotic and abiotic stress (Zhao *et al.*, 2013) and it reduces a number of stresses *via* controlling ion homeostasis, metal transport, antioxidant defense systems, oxidative stress and more. Additionally, it has been discovered that NO interacts with a variety of other signaling molecules, including gibberellins (GA), salicylic acid (SA) and abscisic acid (ABA) (Kumar and Ohri, 2023). In the ABA-induced stomatal closure signaling cascade, NO controls potassium and calcium ion channels to maintain ion homeostasis in guard cells (Agurla *et al.*, 2018). Moreover, NO efficiently stimulates the SA signaling pathway's defense gene expression, strengthening plant resistance to infections (Bellin *et al.*, 2013). In the synthesis of plant gibberellins

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(GA), NO activates the transcription of key enzymes GA3ox1 and GA3ox2, promoting GA synthesis and breaking seed dormancy (Bethke *et al.*, 2007).

NO can directly scavenge ROS or activate antioxidant defense mechanisms in response to non-biological stress circumstances like drought (Sahay and Gupta, 2017). Research has shown that exogenous NO can regulate the physiological and biochemical processes in fragrant rice to reduce the levels of MDA and H_2O_2 generated by cadmium stress (Imran *et al.*, 2023). Ahmad *et al.* (2021) reported that NO in synergy with silicon can reduce the absorption of arsenic in cabbage, thereby alleviating the toxicity of arsenic stress on plants.

SNP is an exogenous NO donor that releases NO when dissolved. S-nitrosylation is the major pathway for the biologically active transfer of NO, where NO partly forms S-nitrosothiol by covalently binding with cysteine thiol in proteins. This chemical change is quickly becoming recognized as a fundamental aspect of plant life and as a model redox-based post-translational modification (Yu *et al.*, 2014). By boosting the activities of superoxide dismutase and catalase, exogenous SNP can decrease the formation of MDA and H_2O_2 in soybeans during salt stress, increasing the soybeans' survival rate (Jasid *et al.*, 2009). Given the increasing trend of potential expansion in the range of drought during crop growth seasons, the combined use of exogenous SNP during irrigation can enhance drought resistance in plants. In this experiment, HN44 and HN65 soybeans were irrigated with varying doses of SNP solution during the flowering stage and NO content, MDA content and leaf area per plant were measured, providing theoretical support for the study of soybean's response to drought stress.

MATERIALS AND METHODS

Drought-tolerant Heinong 44 (HN44) and sensitive Heinong 65 (HN65) are the tested soybean cultivars in this study (Wang *et al.*, 2022).

In the experiment, sand culture was employed and the pots were cylindrical plastic containers with a 20 cm diameter and a 40 cm height. After perforating the bottom, it was covered with a mesh and clean river sand was filled into the pots. Six seeds were sown in each pot and distilled water was irrigated once a day, 500 ml each time, until the unifoliate leaves were fully unfolded. Then, thinning was done, leaving three consistently growing soybean seedlings in each pot. Thereafter, modified Hoagland nutrient solution was irrigated once a day, every time 500 ml, until the flowering stage (R2). Drought and exogenous SNP treatments were performed during the flowering stage. PEG-6000 and SNP were dissolved in the modified Hoagland nutrient solution and each treatment was set as shown in Table 1. Irrigating the nutritional solution with 15% PEG-6000 and SNP once a day, 500 ml each time. Samples were taken on day 1, 3, 5 and 7 of the treatment. Three pots were chosen for each treatment, with three soybean plants selected for each pot. The sampling sites were the second and third inverted leaves of the soybean. The collected soybean leaf samples were divided into two parts: one part was immediately measured after obtaining fresh leaves using an icebox for preservation and for further examination, the remaining portion was quickly frozen in liquid nitrogen and kept in an ultra-low temperature freezer. All measurements were conducted in triplicate.

The colorimetric approach was used to determine the MDA content (Hodges *et al.*, 1999). Following the addition of 1 ml of 10% trichloroacetic acid and 0.1 g of the sample to a mortar, the mixture was ground and centrifuged at 12000 g for 10 minutes. After adding 0.2 ml of 0.67% thiobarbituric acid to the homogenate (0.4 ml), the mixture was heated to 100°C for 30 minutes and the sample was boiled. After cooling, the sample was centrifuged twice to obtain the supernatant and the absorbance at 450, 532 and 600 nm was measured.

With a few minor adjustments, the NO content was determined using Ding *et al.* (1988) methodology as guidance. Using a mortar and pestle, 0.6 g of powdered leaf samples and 3 ml of cold, 50 mM acetic acid buffer (pH 3.6) containing 4% zinc diacetate were combined. The homogenate was centrifuged for 15 minutes at 4°C and 10,000 g to collect the supernatant. The particle was centrifuged after being cleaned with 1 ml of extraction buffer, as previously mentioned. After mixing the two supernatants, 0.1 g of charcoal was added. The filtrate was obtained following vortexing and filtration. 30 minutes were spent incubating a combination of 1 ml of the filtrate and 1 ml of Griess reagent at room temperature. At 540 nm, the absorbance was measured. By comparing the NO content with the $NaNO_2^-$ standard curve, it was determined.

The method of measuring the individual leaf area of soybean plants at the R2 stage was conducted using AutoCAD software.

The experimental data were analyzed and plotted using SPSS 22.0 and Excel 2016 for data analysis and visualization.

RESULTS AND DISCUSSION

Variations in soybean leaf NO content with various SNP treatments

Following treatment for the drought, soybean leaves' NO content rose. In HN44 soybean leaves, the NO content increased with increasing SNP concentration and showed an increasing trend with treatment duration. In HN44,

Table 1: The treatment scheme of flowering test.

Treatment	Drought degree (Concentrations of PEG)	SNP concentration (μmol/L)
CK	0	0
C_1	15%	0
C_2	15%	100
C_3	15%	200
C_4	15%	500
C_5	15%	1000

compared to the CK, the NO content increased by 35.56%, 23.56%, 56.57% and 126.87% on the 1st, 3rd, 5th and 7th days after C_1 treatment, respectively, with a significant increase on the 7th day. After being treated with SNP at concentrations C_3, C_4 and C_5, the NO content in HN44 soybean leaves increased by 0.83%, 11.56% and 25.64%, the C_2 decreases by 1.57%, respectively, on the 3rd day compared to the 1st day. The NO content in the soybean leaves treated for 5 days was 16.92%, 16.04%, 11.39% and 6.04% higher than the NO content in the leaves treated for 3 days, respectively. SNP had an effect at different days and the best effect was observed on the 3rd in HN44 (Table 2).

Although the NO content in C_2 on 5th and 7th day was lower than the C_1 treatment, the NO content in the soybean leaves of HN65 increased as the SNP concentration increased. There was an increasing trend with the increase in treatment days (5 days saw the pinnacle of C_4's initial rise and subsequent decline). In HN65, compared to the CK group, the NO content in the soybean leaves increased by 14.40%, 1.46%, 61.49% and 180.85% on the 1st, 3rd, 5th and 7th day after C_1 treatment, respectively, with the 7th day showing a significant increase. The NO content in the soybean leaves of HN65 treated with SNP at concentrations C_2, C_3, C_4 and C_5 for 5 days was 54.15%, 39.45%, 72.33% and 30.53% higher than that in the leaves treated for 3rd, respectively. The NO content in soybean leaves treated with SNP at concentrations C_2, C_3 and C_5 for 7th days was 3.90%, 7.37% and 15.15% higher than the NO content in the 5th day treatment, respectively. However, C_4 reduced the NO content by 7.12%. SNP had an effect at different days and the best effect was observed on the 5th day in HN65 (Table 2).

During drought stress, the NO concentration rose in HN44 and HN65 soybean leaves, with a faster increase rate in the NO content of HN44 soybean leaves compared to HN65, indicating a rapid response to drought stress. After the exogenous application of SNP, the NO content in both soybean leaf types increased, which is in line with findings of Qu *et al.* (2023). According to Klein *et al.* (2018), adding exogenous nitric oxide to maize greatly raised the amount of nitric oxide in the plant, which had a beneficial impact on oxidative damage in maize. In HN44, the NO content in the leaves after SNP treatment at concentrations C_3 and C_4 on the 5th and 7th day was lower than in the drought group. Following SNP treatment at concentration C_2 on 5th and 7th day, the amount of NO in the leaves of HN65 was lower than that of C_1. The decrease in NO content in soybean leaves under drought stress following treatment with lower concentrations of SNP for a while may be related to the instability and easy breakdown of SNP, or it may be the result of the level of NO increase by SNP weakening with the duration of drought stress.

MDA concentration variations in soybean leaves with various SNP treatments

Compared to the CK treatment, under drought stress, the MDA content in HN44 soybean leaves increased by 13.62%, 21.83%, 28.55% and 28.93% at 1st, 3rd, 5th and 7th, respectively. Significant increases were observed at all time points. The MDA content in HN44 steadily dropped as the SNP concentration rose. The MDA content in the leaves showed a tendency of first reducing and then increasing under the same concentration treatment. Compared to the C_1 treatment, the amount of MDA in the C_5 treatment's leaves decreased by 12.83%, 36.52%, 28.15% and 22.16% on the 1st, 3rd, 5th and 7th day, respectively, with significant differences between the treatments. Under concentrations C_2, C_3, C_4 and C_5, the MDA content in the leaves decreased by 18.26%, 21.99%, 22.60% and 29.24% on the 3rd day compared to the 1st day, respectively. The MDA content in the leaves increased by 12.38%, 7.04%, 6.83% and 16.08% on the 5th compared to the 3rd, respectively. SNP had an effect at different days and the best effect was observed on the 3rd in HN44 (Table 3).

In HN65 soybean leaves under drought stress, the amount of MDA rose by 12.09%, 21.83%, 28.55% and

Table 2: The variations in NO concentration in HN44 and HN65 soybean leaves on various treatment days.

Variety	Treatment	NO contents (nmol·g^{-1}FW)			
		1st	3rd	5th	7th
HN44	CK	177.78±7.64c	232±38.22c	306.78±44.78b	224.89±18.35d
	C_1	241±7.64bc	286.67±5.52bc	480.33±21.06ab	510.22±12.63bc
	C_2	339.56±24.42ab	334.22±40.66bc	390.78±68.73b	427±7.88c
	C_3	373.67±55.77ab	376.78±82.39bc	437.22±37.18b	467.67±10.39bc
	C_4	396.89±15.19a	442.78±71.11ab	493.22±80.12ab	569.89±87.51b
	C_5	471.44±86.51a	592.33±9.01a	628.11±61.98a	762.78±3.31a
HN65	CK	124.22±14.91d	257.89±26.13b	277.56±55.13c	159.56±2.91d
	C_1	142.11±4.19d	261.67±19.68b	448.22±58.79bc	448.11±26.14bc
	C_2	220.67±18.55cd	273.33±39.50b	421.33±24.27bc	437.78±9.16c
	C_3	283±58.14bc	339.67±39.25b	473.67±86.35b	508.56±39.10bc
	C_4	376.33±72.51ab	397.56±87.79b	685.11±15.13a	636.33±90.05b
	C_5	456.44±2.90a	537.11±14.25a	701.11±74.77a	807.33±100.84a

Significant changes between several treatments of the same variety are shown by lowercase letters in the figure ($P<0.05$).

Table 3: The differences in MDA concentration between HN44 and HN65 soybean leaves on different treatment days.

Variety	Treatment	MDA contents (nmol·g^{-1}FW)			
		1st	3rd	5th	7th
HN44	CK	48.86±0.40c	46.80±0.81b	47.39±0.44c	47.145±0.51f
	C_1	55.52±0.42a	53.95±1.34a	55.33±0.56a	66.39±0.16a
	C_2	53.83±0.41a	44.00±0.47c	49.44±1.01b	62.68±0.51b
	C_3	51.75±0.517b	40.37±0.40d	43.21±0.26d	58.72±0.69c
	C_4	49.49±1.22c	38.30±1.23d	40.92±0.34e	54.05±1.32d
	C_5	48.40±0.34c	34.25±0.34e	39.76±0.81e	51.68±0.03e
HN65	CK	49.81±1.07bc	47.98±0.48de	48.25±0.75c	48.11±1.09d
	C_1	55.83±0.55a	58.45±0.30a	62.03±0.76a	73.17±0.46a
	C_2	51.58±0.70b	55.88±0.72b	52.45±0.21b	63.79±1.73b
	C_3	51.20±1.36b	52.94±0.62c	49.24±0.59c	61.02±1.63b
	C_4	49.23±1.07bc	49.18±0.75d	46.32±0.55d	56.78±0.93c
	C_5	47.54±0.89c	46.63±0.75e	43.01±0.64e	50.97±0.65d

Significant changes between several treatments of the same variety are shown by lowercase letters in the figure ($P<0.05$).

52.08% on the 1st, 3rd, 5th and 7th day, in that order, showing a significant increase at each time point. With an increase in SNP concentration, the MDA content gradually decreased. Under the same concentration treatment, the MDA content showed an overall trend of initially decreasing and then increasing. On the 1st, 3rd, 5th and 7th day, the MDA content of the C_5 treatment was significantly lower than that of the C_1 treatment, declining by 14.86%, 20.22%, 30.67% and 30.34%, respectively. The MDA content in the leaves on the 7th day under C_2 concentration significantly increased compared to the 7th day in the C_1 treatment. Under concentrations C_2, C_3, C_4 and C_5, the MDA content in the leaves decreased by 6.15%, 6.99%, 5.82% and 7.77% on the 5th compared to the 3rd, respectively. The MDA content in the leaves increased by 21.62%, 23.92%, 22.58% and 18.51% on the 7th compared to the 5th, respectively. SNP had an effect at different days and the best effect was observed on the 5th in HN65 (Table 3).

Under conditions of drought stress, the MDA content of HN44 and HN65 leaves significantly rose, the MDA level of HN65 leaves was greater, indicating more severe membrane lipid peroxidation. Under different concentrations of SNP treatment, the MDA content of the two soybean leaves decreased to varying degrees. This study's findings are in line with a study by Sundararajan *et al.* (2022) that demonstrated sodium nitroprusside's ability to lower MDA levels in tomato seedlings and increase drought tolerance in tomatoes. When soybeans were treated with varying concentrations of SNP, the MDA level of the two soybean leaves first decreased and subsequently increased as treatment duration increased. The reason for this may be that within a certain period of time, SNP has a mitigating effect on drought stress in HN44 and HN65 soybeans. However, under prolonged drought stress, soybean plants suffer severe damage, cell membranes are compromised and membrane lipid peroxidation becomes more severe. The effect of SNP in alleviating drought stress in soybeans is not significant. Therefore, irrigating soybean plants with SNP can promote an increase in NO content in soybean leaves, effectively reduce the MDA content generated by drought stress, alleviate membrane lipid peroxidation in soybeans and mitigate harmful effects on plant growth.

Variations in soybean plant leaf area per plant following various SNP treatments

Drought treatment reduces the individual leaf area of soybean plants and as the treatment duration increases, the inhibitory effect on leaf area growth becomes stronger. In HN44, compared to CK, the individual leaf area decreased significantly after 1st, 3rd, 5th and 7th days of treatment in C_1, with reductions of 1.02%, 4.34%, 8.16% and 9.86%, respectively. There were significant differences in the individual leaf area of HN44 soybean plants between C_1 and C_3, C_4, C_5 treatments on the 1st, 3rd, 5th and 7th days. With the increase in SNP concentration, the individual leaf area of soybean showed a trend of first increasing and then decreasing. Compared with the C_1 treatment, the individual leaf area of the C_4 treatment increased by 0.77%, 3.57%, 6.07% and 8.04% on the 1st, 3rd, 5th and 7th day, respectively. Among the C_2, C_3, C_4 and C_5 treatments, C_4 had the largest leaf area on the 3rd, 5th and 7th days (Table 4).

In HN65, compared to CK, the individual leaf area decreased by 0.99%, 4.37%, 8.12% and 9.38% on the 1st, 3rd, 5th and 7th days, respectively, after the C_1 treatment. There were significant differences in the individual leaf area of HN65 soybean plants between C_1 and C_3, C_4, C_5 treatments on the 5th and 7th days. With the increase in SNP concentration, the individual leaf area of soybean showed a trend of first increasing and then decreasing. Compared to the C_1 treatment, the individual leaf area of the C_4 treatment increased by 0.808%, 2.97%, 5.42% and 7.19% on the 1st, 3rd, 5th and 7th day, respectively. Among the C_2, C_3, C_4 and C_5 treatments, C_4 had the largest leaf area on the 1st, 3rd, 5th and 7th days (Table 4).

Table 4: The difference of leaf area per plant between HN44 and HN65 under different treatment days.

Variety	Treatment	Leaf area per plant (cm^2)			
		1st	3rd	5th	7th
HN44	CK	173.12±0.32a	182.81±0.23a	191.58±0.74a	197.71±0.75a
	C_1	171.36±0.42c	174.88±0.13d	175.94±0.29e	178.22±0.87e
	C_2	172.23±0.19b	175.41±0.23d	176.34±0.24e	180.45±0.67de
	C_3	172.46±0.18ab	178.22±0.07c	178.36±0.50d	182.82±1.21d
	C_4	172.68±0.02ab	181.13±0.60b	186.62±0.67b	192.54±1.13b
	C_5	172.85±0.08ab	177.48±0.09c	181.44±0.33c	186.66±1.14c
HN65	CK	166.2±1.24a	176.59±1.04a	184.96±1.61a	190.81±1.49a
	C_1	164.56±2.37a	168.88±1.33d	169.94±0.87e	172.91±0.49e
	C_2	165.38±0.66a	169.36±1.15cd	170.68±0.40de	174.32±0.79de
	C_3	165.66±0.61a	172.22±0.40bc	172.92±0.69cd	176.57±1.26d
	C_4	165.9±1.04a	173.89±0.13ab	179.15±0.45b	185.34±0.59b
	C_5	165.36±0.52a	171.32±0.62bcd	175.14±0.74c	180.1±1.11c

Significant changes between several treatments of the same variety are shown by lowercase letters in the figure ($P<0.05$).

Under drought stress, the growth of individual leaf area in both HN44 and HN65 soybeans was suppressed, with a significant decrease in leaf area. As the treatment duration increased, the inhibitory effect became stronger, which is consistent with the findings of the study by Peer *et al.* (2023). The application of exogenous SNP alleviated the inhibition of individual leaf area growth in both soybean varieties to varying degrees. Among them, the C_4 treatment showed the best alleviation effect, which is consistent with the findings of the study by Chavoushi *et al.* (2020).

CONCLUSION

Drought stress significantly increased the contents of NO and MDA and decreased the leaf area per plant of the two cultivars. Treatment with different concentrations of SNP effectively increases leaf NO content, reduces MDA levels, alleviating drought damage in soybean plants and the inhibition of leaf area growth. In practical production, it is recommended to apply a 1000 $\mu mol \cdot g^{-1}$ SNP solution when supplementing water during drought disasters, as it effectively alleviates membrane lipid peroxidation damage and enhances drought resistance.

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Conflict of interest

All authors declared that there is no conflict of interest.

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