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Effect of Sodium Nitroprusside on the Growth and Enzymes Related to Nitrogen Metabolism of Melon Seedlings under Low Temperature Stress

Yongping Zhang [1], Qiannan Diao [1], Yanyan Cao [1], Mengya Shi [2], Hongmei Zhang [1], Haijun Jin [1], Shouyang Jiang [3], Haibin Shen [1]
1 Horticultural Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai Key Laboratory of Protected Horticultural Technology, Shanghai, 201403, China
2 National Agro-Tech Extension and Service Center, Beijing, 100125, China
3 Shanghai Agricultural Scientific Seed and Seeding CO., LTD, Shanghai, 201106, China
Corresponding author email: shb8311@163.com
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Abstract In order to study the effects of 200 μmol/L SNP for 1, 3, 5, 7 d on the growth and the enzymes related to nitrogen metabolism under low temperature stress 12/6℃, day/night, and to explore the feasibility of exogenous SNP regulating low temperature stress on melon (*Cumumis melo.* L) seedlings, the melon seedlings were used as experimental materials and cultivated by substrate culture method. NO donor sodium nitroprusside (SNP) was sprayed on the leaves. The results showed that with the prolongation of low temperature stress, the seedlings growth and nitrogen metabolism were inhibited to some extent. After treatment with exogenous SNP , the seedlings growth (leaf area, shoot fresh weight and root fresh weight) , the contents of nitrate nitrogen, free amino acid, soluble protein and the activities of key enzymes of nitrogen metabolism (NR, NiR, GS, GDH, GOGAT and AS) were all greatly improved; the contents of ammonium nitrogen, the accumulation of H_2O_2 and the production rate of superoxide anion radical (O_2^-) decreased dramatically. In summary, the application of exogenous SNP can effectively alleviate the inhibition of low temperature stress on the growth and nitrogen metabolism, and enhance its low temperature tolerance. The longer the stress, the more obvious the effect.
Keywords Melon; Low temperature stress; Sodium nitroprusside; Nitrogen metabolism

As an important environmental factor during plant growth and development, temperature can affect the geographical distribution and yield quality of plants. Melon (*Cucumis melo* L.) is a typical crop that likes temperature and heat resistance. The optimum temperature for its growth period is 25℃~35℃, and plants grow slowly when the temperature is lower than 12℃, while stems and leaves will be damaged when the temperature is lower than 7℃ (Zhang et al., 2017). In the cultivation process of early spring, the harm of low temperature is often encountered, which leads to the inhibition of growth and development, and seriously restricts the production and planting benefits of melon. Therefore, the study on the technical measures of alleviating the damage caused by low temperature stress by exogenous substances has an important role in solving the loss of production and planting.

Nitrogen is an essential element for plant growth and development, and nitrogen metabolism is one of the important physiological metabolic processes in plants. The N absorption rate and assimilation ability of cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum*) and other crops decreased under low temperature stress, resulting in the relative lack of N nutrients (Liu, 2014, Northwest A&F University, pp.30; Bai et al., 2018). Nitric oxide (NO) is a bioactive signaling molecule that regulates physiological processes of plant growth and development. NO can also be involved in plant defense against various stressors (Qiao and Fan, 2008). Previous studies have shown that exogenous NO can improve the low temperature tolerance of eggplant (https://kns.cnki.net/kcms/detail/46.1068.S.20200826.0914.002.html), high temperature tolerance of Chinese cabbage (Xie et al., 2018) and salt stress tolerance of tomato (Wen et al., 2018) by enhancing the antioxidant protection and osmotic regulation ability of plants. Previous studies have also found that appropriate concentration

of NO can effectively alleviate the damage of salt stress on Dianqie (*Atropa belladonna* L.) (Dai et al., 2020), enhance the activity of key enzymes in nitrogen metabolism, and promote the assimilation of inorganic nitrogen sources. The 80 μmol/L SNP ensured the normal carbon and nitrogen metabolism of pumpkin seedlings under salt stress by increasing the activities of enzymes related to nitrogen metabolism, and improved the ability to resist salt stress (Wu et al., 2017). The effects of exogenous NO on nitrogen metabolism of melon seedlings under low temperature stress have not been reported. Therefore, this study used melon as the material, melon seedlings were sprayed with NO donor nitropurna (SNP) under low temperature stress to study the changing trends of ammonium nitrogen, nitrate nitrogen content and enzyme activities related to nitrogen metabolism. In this study, the mechanism of exogenous SNP alleviating low temperature stress in melon seedlings was explored from the perspective of nitrogen metabolism pathway, providing a theoretical basis for alleviating the growth of melon seedlings under low temperature by exogenous substances.

1 Results and Analysis

1.1 Effects of exogenous SNP on growth of melon seedlings under low temperature stress

Compared with the control (CK), low temperature stress (LT) had little effect on the growth of melon seedlings after 1 to 3 days. With the extension of stress time, the decrease amplitude of leaf area, above ground fresh weight and underground fresh weight increased gradually, and the difference was significant at 5 to 7 days of stress. Leaf area, above ground fresh weight and underground fresh weight decreased by 27.16%, 17.94% and 22.03% compared with CK at 7 days of stress, respectively. The leaf area, above-ground fresh weight and underground fresh weight of exogenous SNP treatment (SNP+LT) were not obvious after 1~3 days, but were significantly higher than that of LT treatment at 5~7 days. In conclusion, leaf spraying SNP alleviated the inhibition of low temperature on the growth of melon seedlings, and the longer the stress time, the more significant the effect was (Table 1).

Table 1 Effects of exogenous SNP on the plant growth of melon seedlings under low temperature stress

Time (d)	Treatment	Leaf area (cm^2)	Shoot fresh weigh (g/plant)	Root fresh weight (g/plant)
1	CK	18.86±1.47a	4.87±0.18a	1.52±0.15a
	LT	17.40±0.95a	4.70±0.20a	1.43±0.13a
	SNP+LT	18.44±0.85a	4.84±0.21a	1.49±0.11a
3	CK	20.27±1.55a	5.47±0.31a	1.71±0.16a
	LT	18.24±0.41a	5.00±0.21a	1.50±0.10a
	SNP+LT	20.73±1.59a	5.25±0.48a	1.70±0.14a
5	CK	24.20±0.53a	6.41±0.28a	1.92±0.17a
	LT	18.31±1.76b	5.62±0.37b	1.58±0.14b
	SNP+LT	21.85±1.32a	6.03±0.33ab	1.88±0.17a
7	CK	26.09±1.71a	7.69±0.42a	2.16±0.21a
	LT	19.00±1.40b	6.31±0.15b	1.69±0.07b
	SNP+LT	23.66±1.30a	6.61±0.55b	1.98±0.12a

Note: CK: control, sprayed with distilled water at room temperature; LT: sprayed with distilled water at low temperature; LT+SNP: sprayed with 200 μmol/L SNP at low temperature; Different small letters in the same time meant significant difference among treatments at 0.05 level

1.2 Effects of exogenous SNP on hydrogen peroxide (H_2O_2) content and superoxide anion ($O_2^{\cdot-}$) production rate of melon seedlings under low temperature stress

As shown in Figure 1, H_2O_2 content and $O_2^{\cdot-}$ production rate of melon seedling leaves under normal temperature treatment (CK) did not change significantly during 1~7 days of treatment. Compared with CK, the H_2O_2 content and $O_2^{\cdot-}$ production rate of low temperature stress (LT) increased significantly, and the increasing range first increased and then decreased with the stress time. Compared with LT, the content of H_2O_2 in LT+SNP treatment decreased by 39.19% and 35.38% at 5 d and 7 d, respectively. There was no significant difference between LT and CK. The maximum reduction of $O_2^{\cdot-}$ production rate was 46.94% at 3 d.

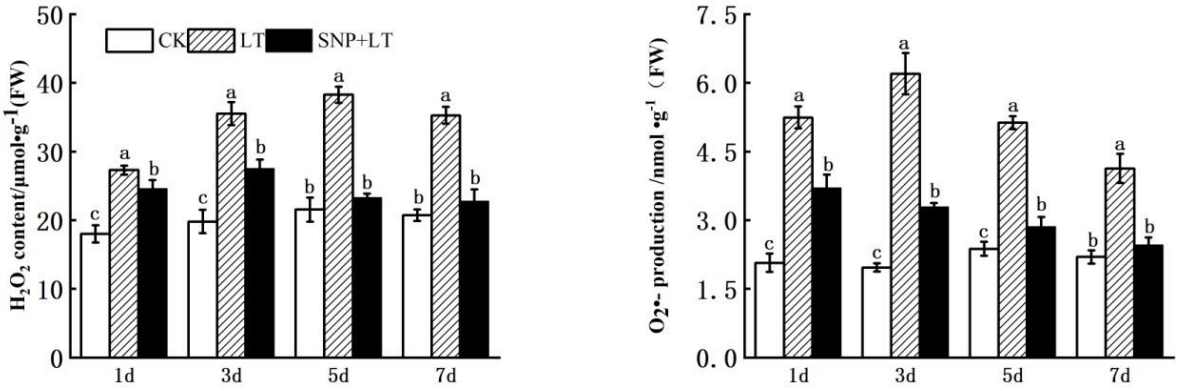

Figure 1 Effects of exogenous SNP on H_2O_2 content and O_2^- production in leaves of melon seedlings under low temperature stress

Note: Different small letters in the same time meant significant difference among treatments at 0.05 level

1.3 Effects of exogenous SNP on nitrogen compounds in melon seedlings under low temperature stress

As shown in Figure 2, compared with the control (CK), low temperature stress (LT) reduced the nitrate nitrogen content in leaves of melon seedlings, and the decrease rate increased with the extension of stress time. On day 1, 3, 5 and 7, it was reduced by 36.63%, 49.15%, 60.83% and 61.61% compared with the control, respectively. The nitrate nitrogen content of SNP (SNP+LT) sprayed with 200 μmol/L at low temperature increased to different degrees compared with the corresponding low temperature stress time, and the increase of 71.78% was the largest at 5 days of stress, but did not reach the control level. At the early stage (1 d) of low temperature stress (LT), the content of ammonium nitrogen in leaves of melon seedlings was significantly decreased compared with the control, and then increased gradually with the extension of stress time, which was 71.23% higher than that of CK at the 7th day of stress. SNP (SNP+LT) sprayed with 200 μmol/L at low temperature increased significantly compared with LT at 1 day of stress, but there was no significant difference with control. However, it decreased significantly in stress 1, 3, 5 and 7 days, by 11.62%, 19.93% and 21.40%, respectively. The contents of free amino acid and soluble protein showed the same trend as nitrate nitrogen content (Figure 2). The leaves of melon seedlings under low temperature stress (LT) were lower than those of control (CK), and the decreasing range increased with the extension of stress time. The content of free amino acids on day 1, 3, 5 and 7 decreased by 24.53%, 37.52%, 48.46% and 51.91% compared with the control, respectively. The soluble protein content at day 1, 3, 5 and 7 was decreased by 31.78%, 47.33%, 55.84% and 67.16% compared with the control, respectively. The contents of SNP (SNP+LT) in 200 μmol/L spray at low temperature increased by different degrees compared with the corresponding low temperature stress time. The content of free amino acid increased by 30.09% at 5 d and soluble protein increased by 51.25% at 3d, but did not reach the control level.

1.4 Effects of exogenous SNP on nitrate reductase (NR) and nitrite Reductase (NiR) activities in leaves of melon seedlings under low temperature stress

As shown in Figure 3, NR and NiR activities of melon seedling leaves under normal temperature treatment (CK) did not change significantly during 1~7 days of treatment. Compared with CK, the activities of NR and NiR under low temperature stress (LT) were significantly decreased (except for 1 day of NiR activity stress), and the decreasing range increased with the extension of stress time, which were decreased by 48.00% and 51.07%, respectively, after 7 days of treatment. Compared with LT, the levels of LT+SNP increased significantly (except for the first day of NiR activity stress), and increased by 20.32% and 60.11%, respectively, at the seventh day of NIR activity stress.

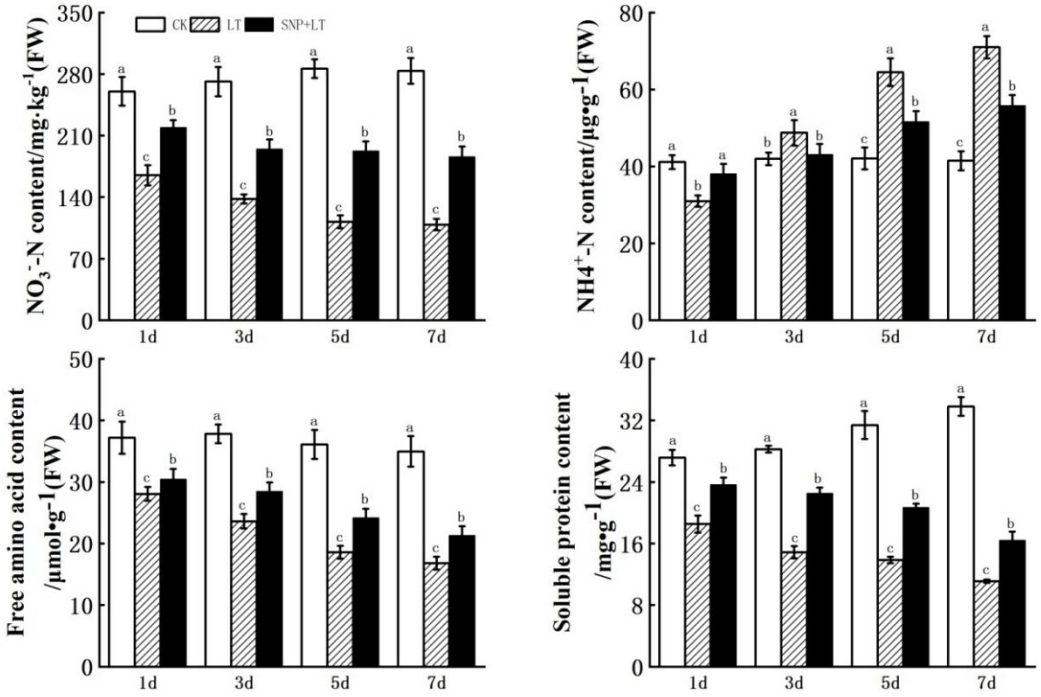

Figure 2 Effects of exogenous SNP on nitrate nitrogen, ammonium nitrogen, free amino acid and soluble proteins content in leaves of melon seedlings under low temperature stress

Note: Different small letters in the same time meant significant difference among treatments at 0.05 level

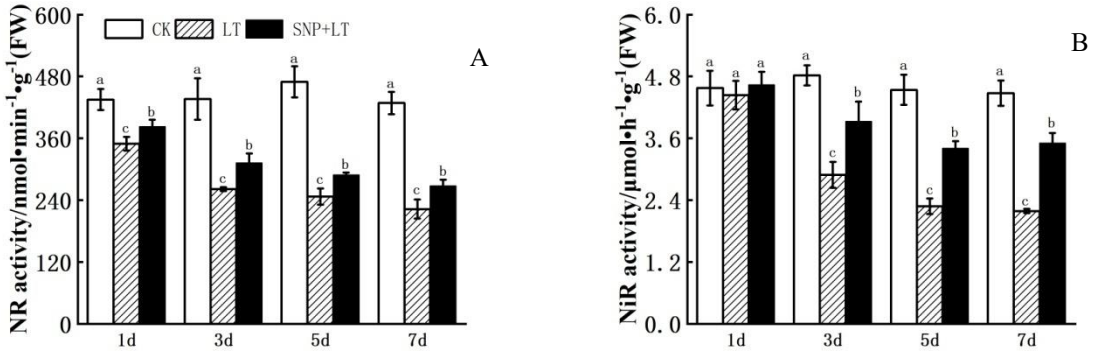

Figure 3 Effects of exogenous SNP on activities of NR and NiR activities in leaves of melon seedlings under low temperature stress

1.5 Effects of exogenous SNP on activities of glutamate synthetase (GOGAT), glutamine synthetase (GS), glutamate dehydrogenase (GDH) and asparagine synthetase (AS) in leaves of melon seedlings under low temperature stress

As can be seen from Figure 4, the activity of GOGAT in leaves of melon seedlings under low temperature stress (LT) was lower than that of control (CK), and the decrease rate increased with the extension of stress time. At 1, 3, 5 and 7 days, it was decreased by 19.843%, 26.02%, 33.72% and 44.18%, respectively. The activity of SNP (SNP+LT) GOGAT at 200 μmol/L spray at low temperature increased significantly compared with the corresponding low temperature stress time, but did not reach the control level and the difference was significant. The activity trends of GS, GDH and AS were basically consistent with that of GOGAT (Figure 4). The activities of three enzymes in the leaves of melon seedlings under low temperature stress (LT) were significantly decreased compared with the control, and the decreasing range increased with the extension of stress time. The activities of three enzymes of SNP (SNP+LT) spraying 200 μmol/L at low temperature were increased compared with the corresponding low temperature stress time. Among them, the activity of GS increased the most after 5 days of stress. The increase rate was 52.12%, while the increase rate of AS activity was the smallest at 5 days of stress, increased by 10.72%. The increase of GDH activity increased gradually with the extension of stress time, but did not reach the control level and the difference was significant.

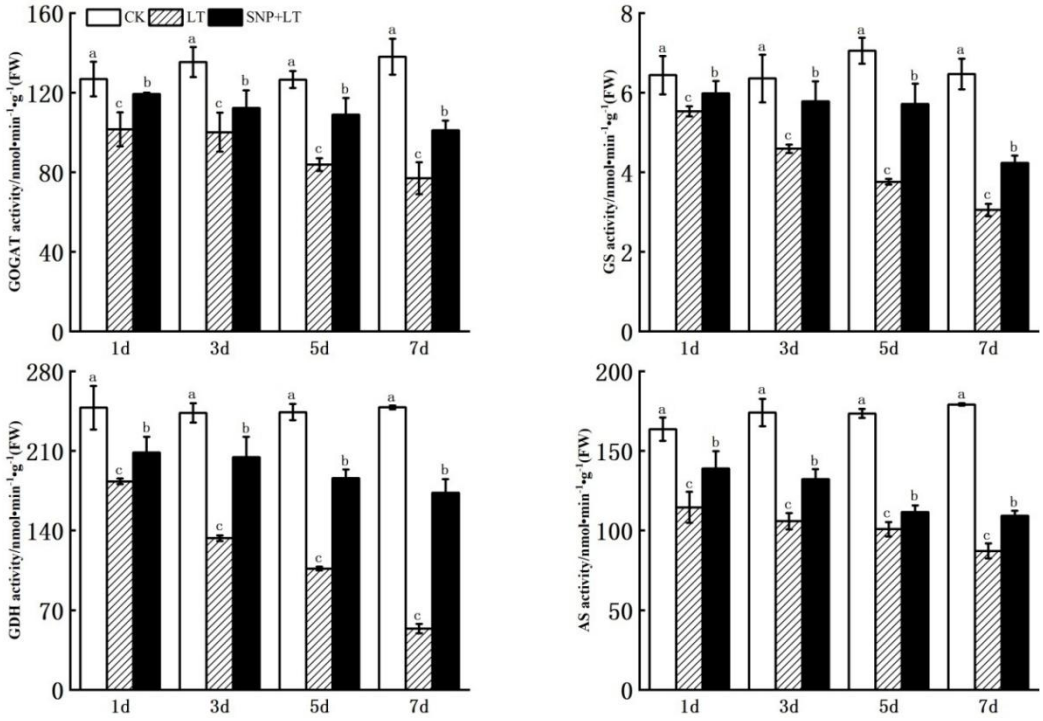

Figure 4 Effects of exogenous SNP on activities of GOGAT, GS, GDH and AS in leaves of melon seedlings under low temperature stress

Note: Different small letters in the same time meant significant difference among treatments at 0.05 level

2 Discussion

Low temperature stress affects crop growth and development, and then leads to decreased yield and quality. As a signaling molecule, NO plays an important regulatory role in plant growth and development, response to stress and programmed cell death (Asgher et al., 2017). The results of this experiment showed that compared with the control, the leaf area, above-ground fresh weight and root fresh weight of melon seedlings were significantly reduced after 5 to 7 days of low temperature stress (Table 1). Zhang et al. (2020) also obtained similar results in the study of eggplant.

Under normal growth conditions, the production and removal of intracellular free radicals are in a dynamic equilibrium state, but when plants are under stress, this balance is broken, resulting in a large amount of accumulation of reactive oxygen species (ROS) such as H_2O_2 content and $O_2^{\cdot -}$ production rate. The level of the total amount directly reflects the degree of cell membrane damage and the relief effect on stress (Zai et al., 2001; Wang et al., 2017). In this study, H_2O_2 content and $O_2^{\cdot -}$ production rate in leaves of melon seedlings were significantly increased under low temperature stress compared with the control, and the increase rate increased with the extension of stress time. After SNP spraying, the H_2O_2 content and $O_2^{\cdot -}$ production rate in the leaves of melon seedlings began to decrease, indicating that exogenous SNP treatment could alleviate the membrane lipid peroxidation damage caused by low temperature stress, and had a certain positive effect on maintaining the normal metabolism of plants. This is consistent with the results of Wen et al. (2018) on tomato and Seckin et al. (2009) on wheat under salt stress.

Nitrogen is one of the important factors affecting plant growth and metabolism. Nitrate nitrogen and ammonium nitrogen are the two main nitrogen sources absorbed and utilized by plants. NO^{3-} is catalyzed by NR and NiR to generate NH^{4+}, and the assimilation of NH^{4+} into organic nitrogen can effectively maintain nitrogen balance and relieve the toxic effect (Herber et al., 2001; Wang et al., 2018). The activity of enzymes related to nitrogen metabolism, such AS NR, NiR, GS, GOGAT and AS, indicated the intensity of N metabolic activity, and temperature significantly affected the activity of N assimilation enzyme in plants. Yao et al. (2013) and Liu (2014, Northwest A&F University, pp.30) found in cucumber and tomato that the activity of nitrogen metabolizing

enzymes decreased with the decrease of treatment temperature, and the process of nitrogen metabolism was inhibited. NR is a regulatory enzyme in plant nitrogen metabolism, and its activity indirectly affects nitrogen conversion. Under salt stress, exogenous SNP can increase the nitrate nitrogen content of *Atropa belladonna* seedlings, induce the increase of NR activity, promote the reduction and assimilation of NO^{3-}, and increase the contents of soluble protein and free amino acid, thus alleviating the damage of salt stress on *Atropa belladonna* seedlings (Dai et al., 2020). GS is a key enzyme in the process of ammonium assimilation, and its activity has a great influence on ammonium nitrogen. GDH can relieve or alleviate the accumulation of ammonium under adverse conditions (Tang et al., 2017). In this study, the contents of nitrate nitrogen, free amino acid, soluble protein and enzyme activities related to nitrogen metabolism in leaves of melon seedlings decreased with the extension of low temperature stress time, while the content of ammonium nitrogen continued to increase, indicating that low temperature stress inhibited the nitrogen metabolism of melon seedlings and affected the growth and development of melon seedlings. After spraying 200 μmol/L SNP, nitrate nitrogen content, free amino acid content, soluble protein content and enzyme activities related to nitrogen metabolism were significantly increased, while ammonium nitrogen content was decreased. Therefore, exogenous SNP can promote the nitrogen metabolism of melon seedlings under low temperature stress, improve the key enzyme activities of nitrogen metabolism, and induce the conversion of inorganic nitrogen to organic nitrogen, thus improving nitrogen-containing organic matter (Xu, 2014).

In conclusion, the growth, contents of nitrate nitrogen, free amino acid, soluble protein and enzyme activities related to nitrogen metabolism were significantly increased after exogenous SNP spray treatment, while the contents of ammonium nitrogen, hydrogen peroxide and superoxide anion production rate were significantly decreased. These results indicated that 200 μmol/L SNP could effectively relieve the inhibition effect of low temperature stress on melon seedlings and improve their low temperature tolerance, and the effect was more obvious with longer stress time.

3 Materials and Methods

3.1 Test materials and test design

The test melon (*Cumumis melo* L.) 'XL-1' was bred independently by Horticulture Research Institute of Shanghai Academy of Agricultural Sciences. The donor of NO was sodium nitroprusside ($[Na_2Fe(CN)_5]$ NO, SNP, Sigma Corporation). The seed after germination was sown in a plastic nutrition bowl with a special watermelon seedling growing medium, and placed in a room at room temperature for seedling culture.

According to the preliminary test results, when the melon seedlings grew to 3 leaves and 1 heart, the plants with the same growth tendency were selected for (1) spraying distilled water at normal temperature as control (CK); (2) Spray distilled water (LT) at low temperature; (3) Four treatments of SNP (SNP+LT) were sprayed with 200 μmol/L at low temperature. 20 plants per treatment, 3 repetitions, spray once a day. The normal temperature was 28℃ at day / 22℃ at night, and the low temperature stress was 12℃ at day / 6℃ at night. All indexes were determined on day 1, 3, 5 and 7.

3.2 Measuring items and methods

3.2.1 Growth index measurement

The blade area was measured by Li-3100 blade area tester (Li-COR Corporation, USA). Rinse the plants with deionized water and blot out the water. The electronic balance with accuracy of 0.01 was used to weigh the fresh weight above ground and the fresh weight below ground. Each treatment was repeated 3 times.

3.2.2 Determination of superoxide anion ($O_2^{\cdot-}$) and H_2O_2 content

The production rate of superoxide anion ($O_2^{\cdot-}$) was determined according to the method of Wang and Luo (1990). Hydrogen peroxide (H_2O_2) was determined by the method of Guo et al. (2015).

3.2.3 Determination of amino acid, soluble protein, ammonium nitrogen and nitrate nitrogen

Soluble protein content was determined by Coomassie Blue Staining G250 method (Zhang et al., 2017). The free amino acid content was determined according to the method of Li (2000, Higher Education Press, pp.167-169).

The contents of nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were determined by the kit of Suzhou Comin Biotechnology Co., LTD.

3.2.4 Determination of enzymes related to nitrogen metabolism

Nitrate reductase (NR) was determined according to Wang et al. (2018) in vitro method. Nitrite reductase (NiR) was determined by the method of Ye et al. (2019). The catalytic production of 1 μmol $NO2^-$ per gram of fresh sample per hour was 1 NR activity unit. Glutamine synthetase (GS), glutamate synthetase (GOGAT), glutamate dehydrogenase (GDH) and asparagine synthetase (AS) were determined by the kit of Suzhou Comin Biotechnology Co., LTD. The production of 1 μg ammonia per gram of fresh sample per hour is defined as a unit of enzyme activity.

3.3 Statistical analysis

Origin7.5 software was used for mapping and SPSS19.0 statistical software was used for multiple comparisons.

Authors' contributions

ZYP and SHB participated in the design of the experiment. ZYP conducted data analysis and paper writing. DQN, CYY, ZHM and JHJ participated in the experimental design and results analysis. SHB made the final revision of the paper. All authors read and approved the final manuscript.

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