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Effect of Exogenous NO on the Plant Growth and Antioxidant System of Eggplant Seedlings under High Temperature Stress

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Abstract Effects of 0.05~5mmol/L sodium nitroprusside (SNP, nitricoxidedonor) on the growth, antioxidant enzyme activities and reactive oxygen metabolism in leaves and growth of eggplant seedlings with spraying exogenous were investigated, using eggplant variety of 'Tewangd' under high-temperature stress ((43±1)°C/(38±1)°C(day/night)). The results showed that: (1) The plant growth was significantly inhibited as well as the chlorophyll content was significantly decreased under high temperature stress. Different concentrations of SNP treatments could enhance the seedlings growth and increase the chlorophyll content, and the most effective treatment is 0.1 mmol/L SNP. (2) The content of malondialdehyde (MDA), superoxide anion ($O2•^{-}$) producing rate and hydrogen peroxide (H2O2) in eggplant seedlings under high temperature stress were significantly decreased after 0.1 mmol/L SNP treatment. Meanwhile, the content of permeable substances (proline, soluble protein, soluble sugar) increased significantly in eggplant seedlings. (3) The superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and ascorbic acid peroxidase (APX), dehydrogenation ascorbic acid reductase (DHAR), single dehydrogenation ascorbic acid reductase (MDHAR), glutathione reductase (GR) activities of antioxidant enzymes and antioxidants such as ascorbic acid (AsA) and glutathione (GSH) contents of eggplant seedlings under high temperature stress, after dealing with the 0.1 mmol/L SNP, were significantly increased, however, the AsA/DHA, GSH/GSSG ratio were reduced. The addition of NO scavenger hemoglobin partially or completely reversed the above effects. In this study, the physiological effects of exogenous NO on the resistance of eggplant to high temperature stress were investigated in order to provide theoretical basis for the application of NO in facility cultivation. This study shows that the activity of antioxidant enzymes were further improved, the osmotic regulation substances contents were increased, the stability of cell membrane system was maintained and the inhibition of seedling growth was repaired under high temperature stress with the exogenous 0.1 mmol/L SNP treatment. This study may provide the theory basis for the exogenous NO application in facility culture.

Keywords Exogenous NO; Eggplant; High temperature stress; Seedling growth; Antioxidation

Eggplant (*Solanum melongena* L.) is a vegetable of *Solanum* genus in the family of Solanaceae, which is widely cultivated all over the world. It is one of the main vegetable species under protected cultivation in China (Lyu et al., 2019). Eggplant originated in India, and China is the second place of origin. The optimum temperature for eggplant growth is 22°C~30°C. If the temperature exceeds 35°C, it will lead to leaf burns, poor development of flower organs, poor coloring of fruit skin, reduction of fruit setting percentage, and increase of deformed fruits (Lian et al., 2017), which will lead to the decline of yield and quality.

Nitric oxide (NO), named "star molecule" by Science in 1992, which is a kind of gas signal molecule with biological activity. It plays an important regulatory role in many physiological processes such as plant seed germination, growth and development, photomorphogenesis and stress defense (Domingos et al., 2015; Asgher et al., 2017), and has attracted much attention in the role of resistance to abiotic stress. Many researchers have found that exogenous NO can improve the tolerance of plants to abiotic stresses such as low temperature, drought and salt stress through a variety of ways, thereby alleviating the oxidative damage of plants under high temperature (Corpas et al., 2011; Fan et al., 2014; Gadelha et al., 2017; Nabi et al., 2019). High temperature stress will cause a large amount of reactive oxygen species (ROS) to be produced in plants, causing oxidative damage to the growth and development of plants. The content of MDA, the O_2•¯ producing rate and the content of H_2O_2 in eggplant pericarp will increase, membrane lipid peroxidation will occur in cells, and the anthocyanin content and the activity of anthocyanin synthesis related enzymes in the pericarp will decrease, leading to the discoloration of the pericarp (Wu et al., 2018). The antioxidation mechanism of enzymes is a key part of the ROS scavenging system. Research results showed that under high temperature stress, exogenous NO could eliminate ROS produced in the body and reduce the content of MDA, the membrane peroxidation product, by regulating the activities of SOD, POD, CAT, APX and other antioxidant enzymes in the ROS scavenging system. Shang and Gao (2015) found that the content of osmotic regulators (Pro, soluble protein, soluble sugar) in plant body increased significantly after exogenous NO treatment, so the osmotic regulation ability of cells was enhanced, and the damage of ROS to plant body was reduced. In addition to the antioxidant enzyme system, the non-enzyme system AsA-GSH cycle will also work with the antioxidant enzyme system to eliminate ROS and improve the plant's ability to withstand high temperature and drought stress under high temperature and drought stress (Han et al., 2018). At present, the effects of exogenous NO on the growth and physiological indexes of eggplant seedlings under high temperature stress have not been reported.

In this experiment, the eggplant variety 'Tewangd', which was independently selected by Shanghai Academy of Agricultural Sciences, was used as the material to study the effects of exogenous NO on the growth and stress resistance of eggplant seedlings under high temperature, and to explore the physiological role of exogenous NO in improving the heat resistance of eggplant under high temperature stress, so as to provide theoretical support for its application in protected vegetable cultivation.

1 Results and Analysis

1.1 Effects of exogenous NO at different concentrations on the growth of eggplant seedlings under high temperature stress

Compared with CK, HT treatment significantly reduced the plant height, stem diameter, shoot fresh weight, root fresh weight and chlorophyll content of eggplant seedlings by 10.67%, 8.89%, 17.22%, 30.95% and 51.55%, respectively after 10 d of high temperature stress (Table 1). Compared with HT, 0.05~5 mmol/L SNP treatment could increase the biomass and chlorophyll content of eggplant seedlings under high temperature stress, and the changes of biomass accumulation of eggplant seedlings under different concentrations of SNP treatment were different, among which 0.1 mmol/L and 0.3 mmol/L SNP treatment had the best effect. The plant height, stem diameter, shoot fresh weight, root fresh weight and chlorophyll content of 0.1 mmol/L SNP treatment were 10.18%, 9.51%, 16.93%, 38.27% and 50.32% higher than those of HT, respectively, while those of 0.3 mmol/L SNP treatment were 9.12%, 9.27%, 15.81%, 20.32% and 36.42% higher than those of HT treatment.

In the following experiments, the SNP concentration with the best spraying effect is 0.1 mmol/L and 0.3 mmol/L, respectively.

Table 1 Effects of exogenous NO on the plant growth of eggplant under high temperature stress

Treatment	Plant height (cm)	Stem diameter (mm)	Shoot fresh weight (g)	Root fresh weight (g)	Chl a+b content (mg/g)
CK	25.30 ± 0.314^{a}	4.50 ± 0.07^{a}	8.12 ± 0.19^{a}	5.31 ± 0.20^{a}	0.0717 ± 0.0016^{a}
HT	22.60 ± 0.55^{d}	4.10 ± 0.07^{c}	6.72 ± 0.19^{d}	3.69 ± 0.17^{e}	0.0347 ± 0.0013^{h}
HT +0.05 SNP	24.62 ± 0.28^{b}	4.44 ± 0.15^{b}	7.78 ± 0.26^{bc}	4.39 ± 0.16^{c}	0.0454 ± 0.0001^{d}
HT +0.1 SNP	24.90 ± 0.31^{ab}	4.49 ± 0.26^{b}	7.86 ± 0.19^{b}	5.07 ± 0.20^{b}	0.0522 ± 0.0002^{b}
HT +0.3 SNP	24.66 ± 0.18^{b}	4.48 ± 0.08^{b}	7.78 ± 0.12^{b}	4.41 ± 0.06^{b}	0.0470 ± 0.0004^{c}
HT +0.5 SNP	24.00 ± 0.14^{c}	4.08 ± 0.08^{c}	7.15 ± 0.17^{c}	3.97 ± 0.04^{d}	0.0424 ± 0.0003^{e}
HT +1 SNP	23.86 ± 0.30^{c}	4.04 ± 0.11^{c}	7.05 ± 0.11^{c}	3.96 ± 0.03^{d}	0.0397 ± 0.0009^{f}
HT +5 SNP	23.20 ± 0.29^{d}	3.96 ± 0.11^{c}	6.72 ± 0.19^{d}	3.71 ± 0.12^{e}	0.0377 ± 0.0001^{g}

Note: Different small letters in the same column show significant difference at 0.05 level ($P<0.05$)

1.2 Effects of exogenous NO treatment on MDA content, $O_2•^-$ producing rate and H_2O_2 content of eggplant seedlings under high temperature stress

Compared with the control (S_0), after 10 d of high temperature treatment (S_1) alone, MDA content, O_2 producing rate and H_2O_2 content in eggplant leaves were significantly increased by 157.43%, 91.99% and 203.01%, respectively (Figure 1A; Figure 1B; Figure 1C). 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) significantly reduced the content of the above three substances under high temperature stress. The $O_2•^-$ producing rate of S_2 treatment was significantly lower than that of S_4 treatment, and the MDA and H_2O_2 content had no significant change. Under the NO scavenger hemoglobin (Hb) treatment (S_3, S_5), MDA content, $O_2•^-$ producing rate and H_2O_2 content were significantly increased. However, compared with the single high temperature treatment (S_1), they were still significantly reduced, indicating that the mitigation effect of SNP was not eliminated completely.

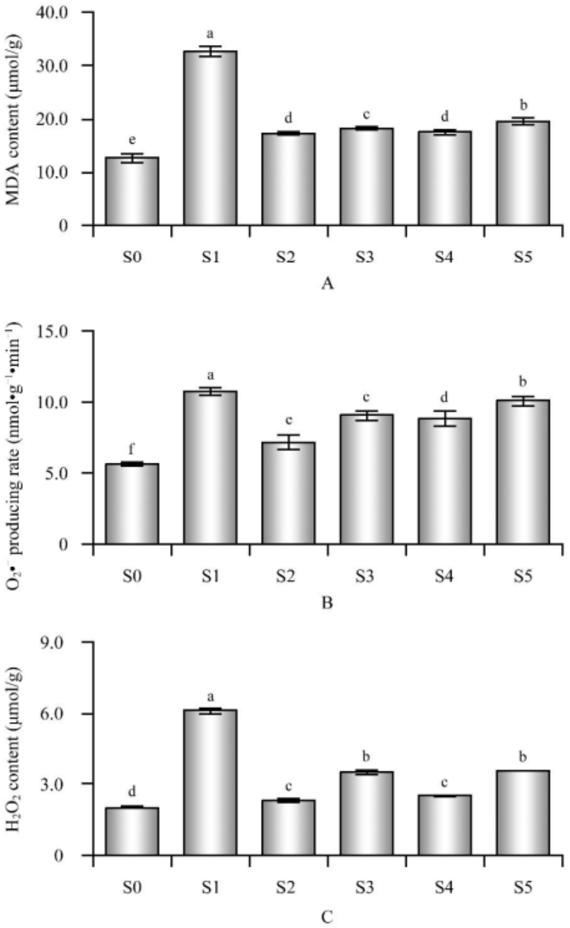

Figure 1 Effects of exogenous NO on MDA contents, $O_2•^-$ producing rate and H_2O_2 contents of eggplant leaves under high temperature stress

Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in MDA contents, B: Changes in $O_2•^-$ producing rate, C: Changes in H_2O_2 contents

1.3 Effects of exogenous NO on SOD, POD and CAT activity of eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0) treatment, the activities of SOD, POD and CAT increased significantly by 44.59%, 14.10% and 106.67%, respectively after high temperature treatment (S_1) alone for 10 d (Figure 2A; Figure 2B; Figure 2C). Compared with S_1, 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) treatments further increased the activities of the three enzymes under high temperature stress, and those of S_2 treatment were significantly higher than those of S_4 treatment. The addition of NO scavenger Hb (S_3, S_5) significantly reduced the activity of all three enzymes, but it was still higher than that of S_1 treatment.

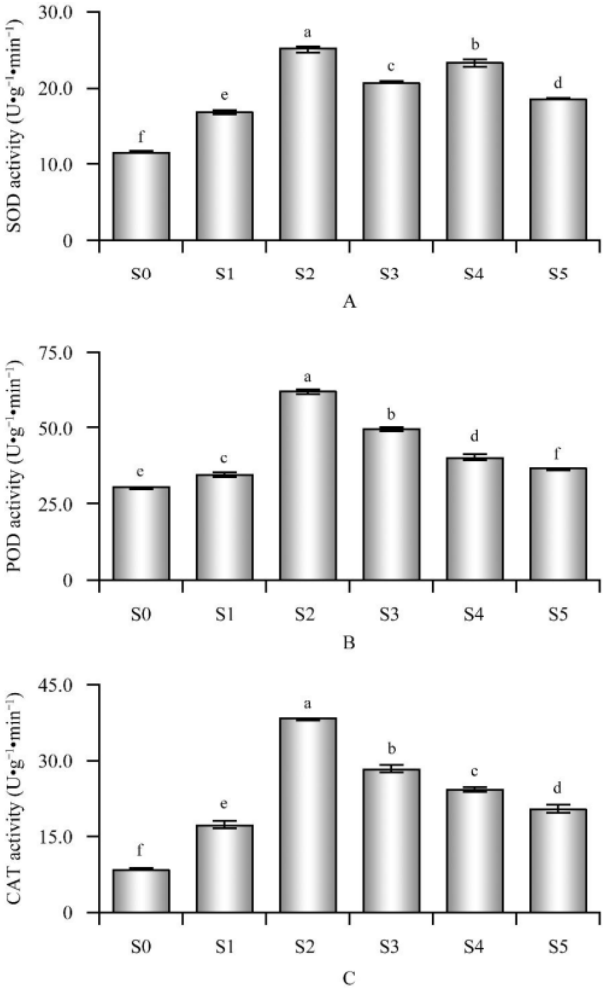

Figure 2 Effects of exogenous NO on SOD, POD and CAT activities of eggplant leaves under high temperature stress

Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in SOD activity; B: Changes in POD activity; C: Changes in CAT activity

1.4 Effects of exogenous NO on the APX, DHAR, MDHAR and GR enzymes activities in eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0), the activities of APX, DHAR, MDHAR and GR were significantly increased by 36.48%, 58.87%, 20.89% and 38.17%, respectively after high temperature treatment (S_1) alone for 10 d (Figure 3A; Figure 3B; Figure 3C; Figure 3D). Compared with S_1, the four enzyme activities of 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) treatments were significantly increased, and the enzyme activities of APX, DHAR, MDHAR and GR in S_2 treatment were increased by 58.31%, 350.11%, 29.09% and 35.31%, respectively. S_4 treatment increased by 22.78%, 361.21%, 4.84% and 28.87%, respectively. Compared with S_2 and S_4, the activities of four enzymes in the treatment with NO inhibitor Hb (S_3, S_5) were significantly reduced, and the MDHAR activity in the treatment with S_4 was still significantly increased, except that there was no significant change in the treatment with S_4 compared with S_1.

1.5 Effects of exogenous NO on DHAR, MDHAR and GR gene expressions of eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0) treatment, the DHAR, MDHAR and GR gene expression levels were significantly increased after 10 days of high temperature treatment (S_1) alone. The expression levels of three genes in 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) treatment were significantly higher than those in S_1 treatment. The addition of NO inhibitors Hb (S_3, S_5) significantly reversed this effect (Figure 4A; Figure 4B; Figure 4C).

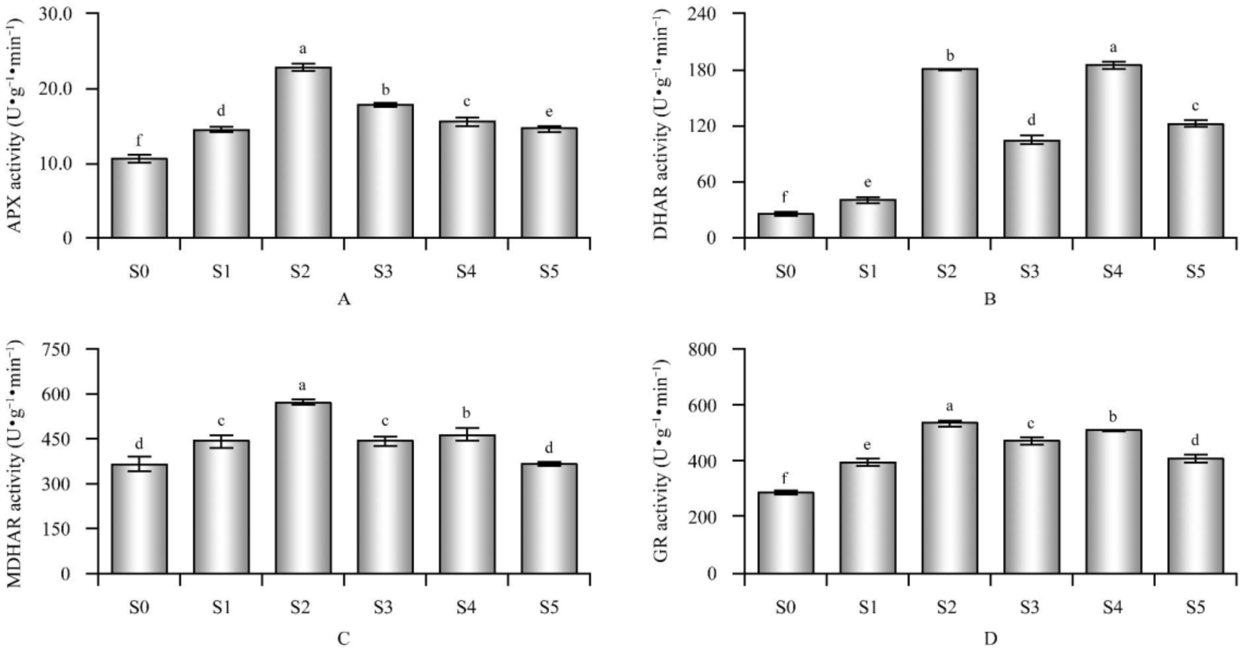

Figure 3 Effects of exogenous NO on APX, DHAR, MDHAR and GR activities of eggplant leaves under high temperature stress
Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in APX activity; B: Changes in DHAR activity; C: Changes in MDHAR activity; D: Changes in GR activity

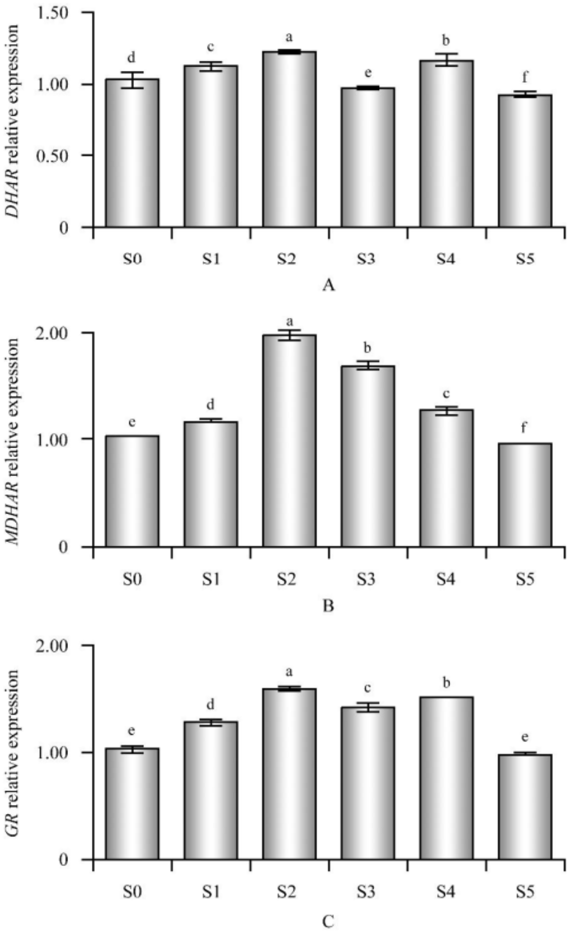

Figure 4 Effects of exogenous NO on DHAR, MDHAR and GR gene expressions of eggplant leaves under high temperature stress
Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in DHAR gene expression; B: Changes in MDHAR gene expression; C: Changes in GR gene expression

1.6 Effects of exogenous NO on AsA, DHA content and AsA/DHA ratio of eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0), the content of AsA and DHA increased significantly, and the ratio of AsA/DHA decreased significantly after 10 d of high temperature treatment (S_1) alone. The treatment of 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) increased AsA content and AsA/DHA ratio, but decreased DHA content under high temperature stress (Figure 5A; Figure 5B; Figure 5C). After adding NO inhibitor Hb (S_3, S_5), AsA content and AsA/DHA ratio decreased, while DHA content increased.

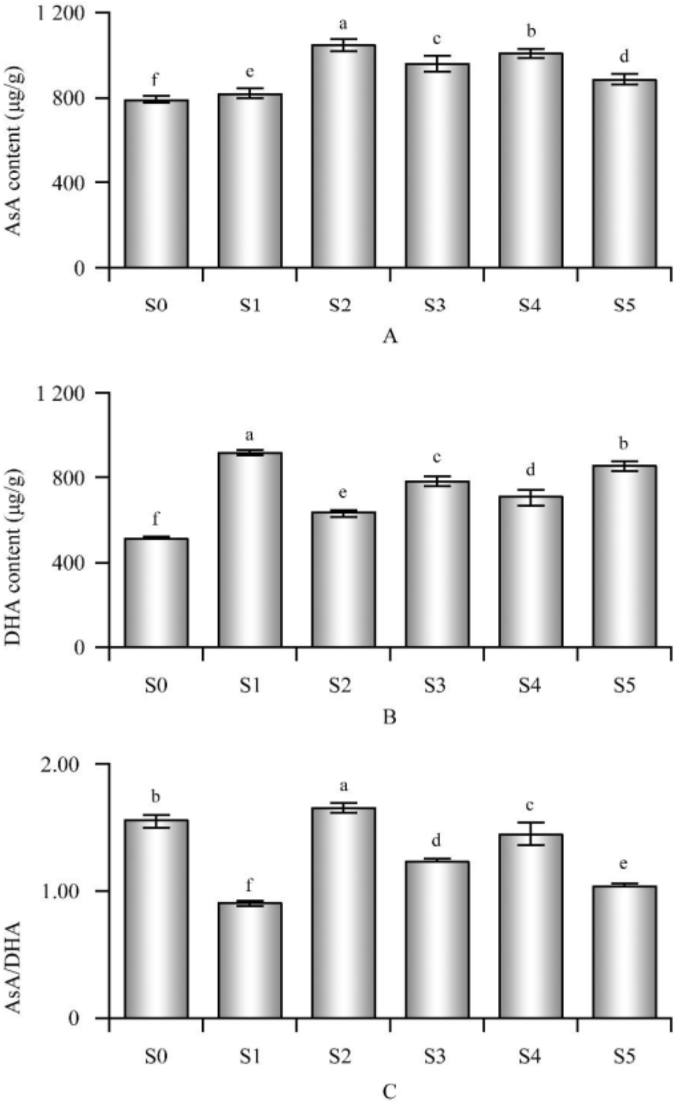

Figure 5 Effects of exogenous NO on AsA, DHA, contents and AsA/DHA of eggplant leaves under high temperature stress

Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in AsA content, B: Changes in DHA content, C: Changes in AsA/DHA

1.7 Effects of exogenous NO on GSH, GSSG contents and GSH/GSSG ratio of eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0), high temperature treatment (S_1) alone for 10 d significantly increased the content of GSH and GSSG in eggplant seedling leaves, while the GSH/GSSG ratio decreased significantly. The treatment of 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) increased GSH content, GSH/GSSG ratio and decreased GSSG content under high temperature stress. After using NO inhibitor hemoglobin Hb (S_3, S_5), GSH content, GSH/GSSG ratio decreased, and GSSG content increased (Figure 6A; Figure 6B; Figure 6C).

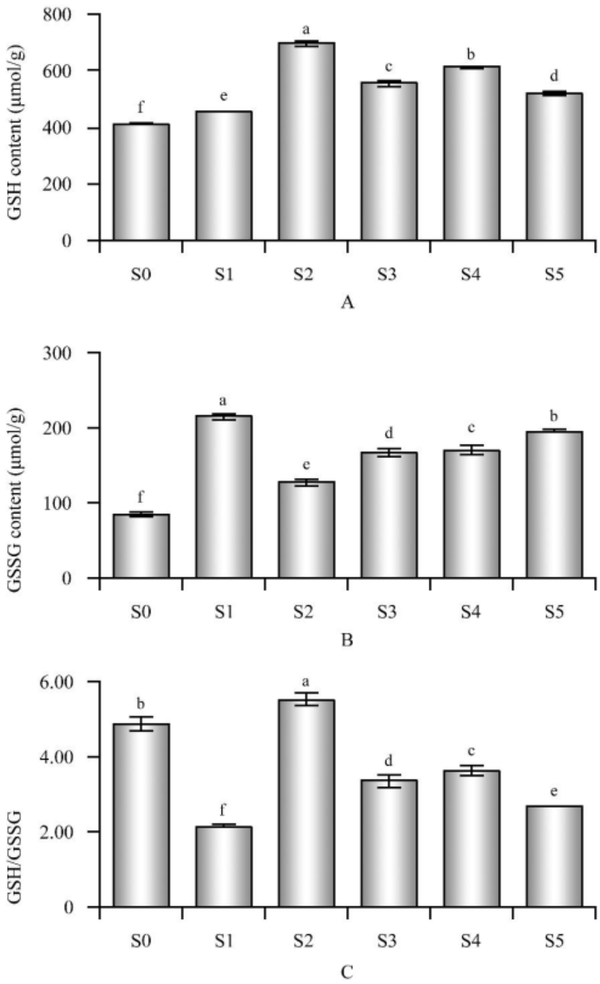

Figure 6 Effects of exogenous NO on GSH, GSSG contents and GSH/GSSG of eggplant leaves under high temperature stress
Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in GSH content; B: Changes in GSSG content; C: Changes in GSH/GSSG

1.8 Effects of exogenous NO on the contents of proline, soluble sugar and soluble protein in eggplant seedlings under high temperature stress

Compared with the normal temperature control (S_0) treatment, the contents of proline, soluble sugar and soluble protein in the high temperature treatment (S_1) alone after 10 d were significantly increased by 10.37%, 8.59% and 2.69%, respectively. The contents of three substances in 0.1 mmol/L SNP (S_2) and 0.3 mmol/L SNP (S_4) treatments were significantly higher than those in S_1 treatment, and those in S_2 treatment were significantly higher than those in S_4 treatment (Figure 7A; Figure 7B; Figure 7C). After NO inhibitor treatment (S_3, S_5), the accumulation of three substances decreased significantly. Compared with S_1 treatment, the contents of all substances in S_3 treatment were significantly increased, and the soluble protein content in S_5 treatment was significantly increased, while Pro and soluble sugar had no significant difference.

2 Discussion

2.1 Effects of exogenous NO on the growth of eggplant seedlings under high temperature stress

Temperature is one of the important environmental factors in the process of plant growth and development. Under high temperature stress, the normal growth and development of plants are inhibited, and the physiological metabolism process is damaged, resulting in the decline of quality and yield, even death (Sun et al., 2010). As an important signal molecule, NO is widely involved in various physiological regulation processes of plants. As a donor of exogenous NO, SNP is used to alleviate the damage of plants caused by various abiotic stresses. Zhang et al. (2020) found that different concentrations of SNP could promote the increase of dry weight of eggplant seedlings under low temperature stress, with the maximum increase at a concentration of 0.1 mmol/L. Sun et al. (2019) reported 50~800 μmol/L SNP treatment could alleviate the growth of tomato seedlings under NaCl stress, with 0.1 mmol/L SNP treatment showing the most significant alleviation effect. This experiment showed that high temperature stress inhibited the growth of eggplant seedlings and reduced the content of chlorophyll, and exogenous NO donor SNP could promote the growth of eggplant seedlings under high temperature stress and delay the degradation of chlorophyll, among which 0.1 mmol/L SNP and 0.3 mmol/L SNP were the best.

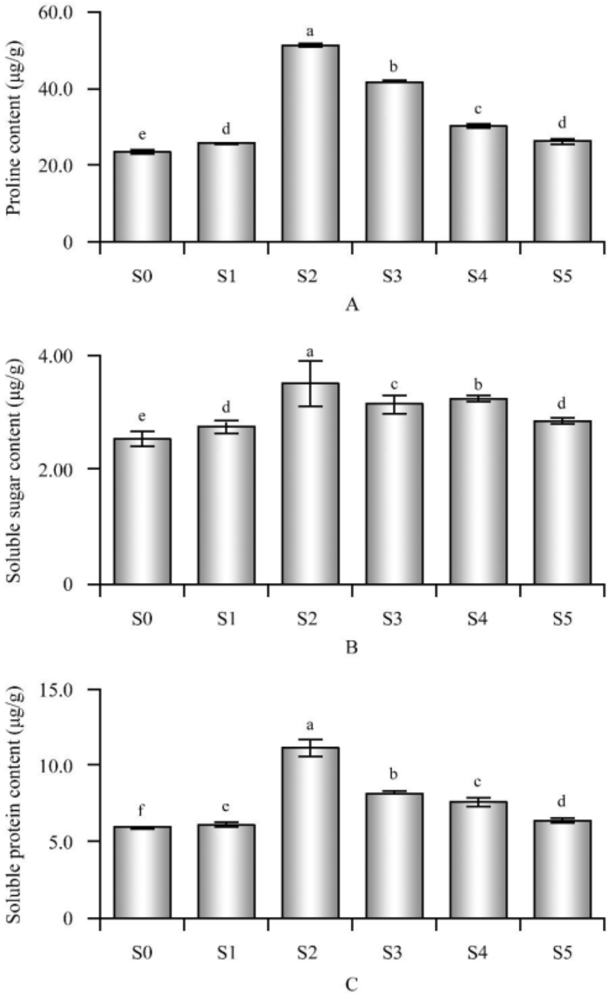

Figure 7 Effects of exogenous NO on the proline, soluble sugar and soluble protein contents of eggplant seedlings leaves under high temperature stress

Note: Different small letters in the same column show significant difference at 0.05 level. A: Changes in proline content; B: Changes in soluble sugar content; C: Changes in soluble protein content

2.2 Effects of exogenous NO on physiological and biochemical characteristics of eggplant seedlings under high temperature stress

Under high temperature stress, membrane lipid peroxidation occurs in plant cells, membrane system is destroyed, and plant growth and development are inhibited. High temperature stress will lead to the formation of ionic hydroxyl radical (-OH) and superoxide anion ($O_2\bullet^-$), molecular singlet oxygen (O^2) and hydrogen peroxide (H_2O_2) and other reactive substances to form peroxidation reactions on unsaturated fatty acids in plant membrane lipids, which in turn leads to the accumulation of MDA in plants and the peroxidation of membrane lipids in cell membranes, and the MDA content, $O_2\bullet^-$ production rate and H_2O_2 content reflect the degree of peroxidation of plant cell membrane lipids (Han et al., 2010). Yang et al. (2011) found that under high temperature stress, 0.2 mmol/L SNP treatment could significantly inhibit the increase of MDA content, $O_2\bullet^-$ production rate and H_2O_2 content in chrysanthemum and alleviate the oxidative damage of chrysanthemum under high temperature stress. In this experiment, the MDA content, $O_2\bullet^-$ production rate and H_2O_2 content of eggplant seedlings were significantly increased under high temperature stress. The content of three substances in eggplant plants began to decrease after the application of SNP (0.1 mmol/L and 0.3 mmol/L), indicating that exogenous NO treatment can alleviate the damage caused by high temperature stress, actively maintain the normal metabolism of plants, and enhance the stress resistance of plants. The NO scavenger hemoglobin promoted the increase of MDA, $O_2 \bullet^{-}$ production rate and H_2O_2 accumulation in eggplant leaves under high temperature stress, further proving that exogenous NO had a certain regulatory effect on the antioxidation of eggplant under high temperature stress.

2.3 Effects of exogenous NO on osmotic regulators in eggplant seedlings under high temperature stress

As important osmoregulation substances in plants, proline, soluble protein and soluble sugar all play an important role in plant stress (Wang et al., 2015). Various protein spatial configurations in plants can be destroyed by high temperature, which leads to protein synthase denaturation, passivation, and inhibition of protein synthesis, causing physiological damage to plants. At this time, plants will accumulate proline, soluble protein, and soluble sugar in large quantities through osmotic adjustment, thus alleviating the physiological damage caused by high temperature (Li et al., 2018). The results of Shang and Gao (2015) showed that exogenous NO could significantly increase the content of proline and soluble protein in the leaves of pepper seedlings under high temperature stress, so as to maintain cell osmotic balance and reduce the damage of cell plasma membrane. The results of this experiment also showed that the contents of Pro, soluble protein and soluble sugar in leaves of eggplant seedlings under high temperature stress were significantly increased after a certain concentration of exogenous SNP treatment. It can be concluded that the osmotic adjustment ability of eggplant seedlings treated with exogenous NO was improved, and the degree of lipid peroxidation of cell membrane was weakened, which alleviated the oxidative damage caused by high temperature to eggplant. The induction effect of SNP was significantly reversed by the addition of the NO scavenger hemoglobin.

2.4 Effects of exogenous NO on AsA-GSH cyclic system of eggplant seedlings under high temperature stress

As important non-enzymatic antioxidants in the AsA-GSH cycle in plants, AsA and GSH can directly react with ROS to generate oxidized DHA and GSSG, which are then reduced to AsA and GSH by DHAR and GR. High temperature stress increased the content of AsA and GSH in plants, which enhanced the stress resistance of plants themselves (Sun et al., 2015). In this study, high temperature stress significantly increased the content of AsA, DHA, GSH and GSSG in eggplant seedlings, resulting in a significant decrease in AsA/DHA and GSH/GSSG ratios. The increase of AsA and GSH content may be a self-response of eggplant to adapt to high temperature stress, and the stress resistance of eggplant can be enhanced by accelerating the formation of both. The application of exogenous NO promoted the increase of ASA and GSH content, AsA/DHA and GSH/GSSG ratio and decreased the content of DHA and GSSG under high temperature stress; the application of NO scavenger hemoglobin reversed this effect. It shows that exogenous NO can promote the regeneration of AsA and GSH under high temperature stress, increase the ratio of reducing state to oxidizing state, cooperate with antioxidant enzyme system, eliminate ROS, reduce membrane lipid peroxidation damage, and maintain the stability of cell membrane. The results are consistent with those of Li et al. (2014).

2.5 Effects of exogenous NO on antioxidant enzymes activities of eggplant seedlings under high temperature stress

In the stress environment, ROS in plants will accumulate excessively to cause damage. There are two kinds of active oxygen radical scavenging systems in plants, namely enzymatic (SOD, POD and CAT) and non-enzymatic (AsA-GSH cyclic system), which play an important role in scavenging active oxygen radicals and maintaining the integrity of membrane structure and function (Fan et al., 2014; Gadelha et al., 2017). $O_2 \bullet^{-}$ was catalyzed by antioxidant enzyme SOD to generate H_2O_2 and O_2, which are then reduced to H_2O and O_2 by POD and CAT (Sun et al., 2019; Talbi et al., 2015). The results of this experiment showed that the activities of SOD, POD and CAT enzymes increased significantly under high temperature stress, which was an active protective mechanism for plants to adapt to the environment after stress. Spraying exogenous NO further significantly increased SOD, POD and CAT in leaves, indicating that exogenous NO could eliminate ROS produced by high temperature stress by inducing enhanced antioxidant enzyme activity, reduce cell oxidative damage, and improve the ability of eggplant to resist high temperature. This is similar to the research results of Yang et al. (2011) on chrysanthemum. The activities of SOD, POD and CAT began to decrease after the application of NO scavenger hemoglobin, and the alleviative effect of SNP was reversed.

Four key enzymes in the AsA-GSH circulatory system: APX, DHAR, MDHAR, GR, eliminate ROS and maintain the stability of cell membrane under the synergistic effect of antioxidant substances (Wu et al., 2015). APX uses AsA to reduce H_2O_2 to H_2O, thus scavenging the toxicity of H_2O_2. DHAR and MDHAR activities catalyze the regeneration of AsA and keep AsA in a higher reduced state in plant tissues. GR can convert GSSG to GSH. The application of NO significantly increased the GR activity. GSSG was converted into GSH to maintain the homeostasis of the intracellular environment, induce the expression of antioxidant enzyme genes, and improve the enzyme activity (Uchida et al., 2002). In this experiment, high temperature stress promoted the significant increase of APX, DHAR, MDHAR, GR activities, and induced the up regulation of DHAR, MDHAR, and GR gene expression in eggplant, indicating that under high temperature stress, plant cells started ROS free radical scavenging system, and induced the enhancement of antioxidant enzyme activities, thus enhancing the tolerance of seedlings to high temperature stress. Under the treatment of exogenous NO, the antioxidant enzyme activity of eggplant seedlings under high temperature stress significantly increased, and the relative expression of DHAR, MDHAR and GR genes also increased to varying degrees, indicating that exogenous NO mainly enhanced the stress resistance ability of eggplant seedlings under high temperature stress by regulating various antioxidant enzyme activities, and also participated in the initiation of antioxidant defense system under high temperature stress at the molecular level. The activities of APX, DHAR, MDHAR and GR decreased after the application of NO scavenger hemoglobin, which reduced the tolerance of eggplant seedlings to high temperature stress.

It can be concluded that exogenous NO treatment can effectively alleviate the damage of high temperature stress on eggplant seedlings and improve their heat tolerance, and 0.1 mmol/L SNP treatment is the best. Under high temperature stress, the growth of eggplant seedlings treated with 0.1 mmol/L and 0.3 mmol/L SNP was promoted, the MDA content, $O_2\bullet^-$ production rate and H_2O_2 content were significantly reduced, and the activities of antioxidant enzymes SOD, POD, CAT, APX, DHAR and GR as well as the antioxidant substances AsA and GSH content were significantly increased. Therefore, the tolerance of eggplant seedlings to high temperature stress was enhanced and the damage suffered was reduced, indicating that the content of osmoregulatory substances and cellular osmoregulatory capacity of eggplant seedlings were significantly enhanced under the treatment with suitable concentrations of SNP to induce the synergistic effect of enzymatic activity of antioxidant system and non-enzymatic AsA-GSH cycle to mitigate the oxidative damage to the plant body by high temperature stress, and the ability of eggplant seedlings to resist high temperature adversity was improved and heat tolerance was enhanced, providing a theoretical basis for further analysis of the molecular mechanism of exogenous NO treatment to improve the heat tolerance of eggplant.

3 Materials and Methods

3.1 Materials

The eggplant variety 'Tewangd' was provided by Shanghai Academy of Agricultural Sciences. NO donor sodium nitroprusside (Sodium nitroprusside, SNP, produced in Sigma) is now used and prepared. Hemoglobins is produced by Sigma.

3.2 Test treatment

Take plump, uniform and pest free seeds, soak them in warm water at 55°C for germination, and then plant them in 32 holes of a disc with vermiculite as the substrate (1 seed/hole). When the seedlings grow to 3~4 real leaves, select the seedlings with the same growth vigor and place them at four light incubators with the temperature of 27°C/22°C (day/night) and light intensity of 400 $\mu mol\cdot m^{-2}\cdot s^{-1}$ or so, respectively, for adaptive cultivation, and treatment was carried out 2 d later.

3.3 Effects of different concentrations of SNP on the growth and chlorophyll content of eggplant seedlings under high temperature, and screening of appropriate SNP concentrations

Experiment I was designed with 8 treatments, (1) Control, normal temperature, with 27°C/22°C (day/night); (2) High temperature, (43±1)°C/(38±1)°C (day/night); (3) High temperature+0.05 mmol/L SNP; (4) High temperature+0.1 mmol/L SNP; (5) High temperature+0.3 mmol/L SNP; (6) High temperature+0.5 mmol/L SNP; (7) High temperature+1 mmol/L SNP; (8) High temperature+5 mmol/L SNP. After 10 d of treatment, the growth and chlorophyll content of eggplant seedlings were determined to screen the best treatment concentration.

3.4 Effects of exogenous SNP on growth, antioxidant enzyme activity and gene expression of eggplant seedlings under high temperature

According to the results of experiment I, 0.1 mmol/L and 0.3 mmol/L SNP were selected to have the best effect on alleviating the growth damage of eggplant seedlings under high temperature stress. On this basis, experiment II was set with 6 treatments. (1) Control, normal temperature, with 27°C/22°C (day/night), denoted by S0; (2) High temperature, (43±1)°C/(38±1)°C (day/night), S1; (3) High temperature+0.1 mmol/L SNP, S2; (4) High temperature+0.1 mmol/L SNP+0.1%(W/V) Hb (Hemoglobin, NO Scavenger), S3; (5) High temperature+0.3 mmol/L SNP, S4; (6) High temperature alone+0.3 mmol/L SNP+0.3% Hb, S5. After 10 d of treatment, leaves with the same growth vigor were selected from eggplants of each treatment group to determine the relevant physiological indicators.

3.5 Determination of seedling growth index

The plant height (cotyledon node to growth point), stem diameter, fresh weight of shoot and fresh weight of root system of eggplant seedlings were measured with ruler, vernier caliper and electronic balance, respectively.

3.6 Determination of physiological and biochemical indexes of seedlings

The determination methods of chlorophyll content, MDA content, production rate of $O_2 \cdot^-$, SOD, POD, CAT, APX activity, Pro content and soluble protein content refer to the methods of Wu et al. (2006). The content of H_2O_2, AsA, DHA, GSH and GSSG were determined with the method of Guo et al. (2015). DHAR, MDHAR and GR activities were determined with the method of Wu et al. (2015).

3.7 RNA extraction and fluorescence quantitative PCR analysis

The total RNA of eggplant leaves from different treatments of experiment II was extracted separately (Total Plant RNA Extraction Kit Omega, USA), and its purity was detected by 1% agarose gel electrophoresis. The cDNA was synthesized using the reverse transcription kit PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa) for subsequent experiments.

Real-time fluorescence quantitative PCR reactions were performed on 7500 PCR instrument of Applied Biosystems Inc., USA, with the reagent of SYBR Premix Ex TaqTM (Perfect Real Time), 20 μL reaction system of SYBR Premix Ex TaqTM (2x) 10 μL, cDNA 2.5 μL, forward and reverse primers (10 μmol/L) 1.5 μL for each, dd H_2O 4.1 μL, 50×ROX Reference Dye 0.4 μL. Repeat 3 times for each sample.

qPCR reaction condition was as follows: pre denaturation at 95°C for 2 min; Denaturation at 95°C for 15 s, 62°C for 32 s, 40 cycles. The dissolution curve of each sample was analyzed to determine the specific amplification of the primer. Action was the internal reference gene. The control treatment (treatment at 25°C for 3 h) was selected as the parameter, and $2^{-\Delta\Delta CT}$ (2 means amplification efficiency) was used to calculate the relative gene expression.

The target genes were *DHAR*, *GR* and *MDHAR*, and the internal reference gene combination was *18srRNA* (Table 2).

3.8 Data analysis

Original7.5 was used for drawing, and Duncan's multiple range test was used for statistical analysis and multiple comparison through SPSS19.0.

Table 2 Primers of target genes used in the quantitative RT-PCR

Gene name	F-primer sequence (5'-3')	P-primer sequence (5'-3')
DHAR	GAAGTGGAGTGTGCCTGAAA	CGTACTTCTCTTCAGCCTTGG
MDHAR	TCCGAACAAACATACCTGGA	GTGTGCGTGTGTGCAGTTAG
GR	TTGGTGGAACGTGTGTTCTT	TCTCATTCACTTCCCATCCA
18sRNA	TAGTTGGACTTTGGGATGGC	AGAGCGTAGGCTTGCTTTGA

Authors' contributions

WXX completed the data analysis and the first draft of the paper. WY, JJ, SHB and TSB prepared experimental materials and participated in the experimental process. WXX and ZSD were the designers and directors of this study, guiding the writing and revision of this paper. All authors read and approved the final manuscript.

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