Front. Agric. China 2007, 1(3): 308–314
DOI 10.1007/s11703-007-0052-5

RESEARCH ARTICLE

FAN Huaifu, GUO Shirong, JIAO Yansheng, ZHANG Runhua, LI Juan

Effects of exogenous nitric oxide on growth, active oxygen species metabolism, and photosynthetic characteristics in cucumber seedlings under NaCl stress

Abstract The study was conducted by means of nutrient solution culture to investigate the effects of exogenous nitric oxide (NO) on growth of cucumber seedlings, active oxygen species metabolism and photosynthetic characteristics in cucumber leaves under 50 mmol/L NaCl stress. The results showed that 10–400 μmol/L exogenous sodium nitroprusside (SNP), especially 100 μmol/L SNP, significantly alleviated the injury to seedlings and increased seedling growth. The activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and the contents of photosynthetic pigments and proline also increased under 50 mmol/L NaCl stress. Similarly, net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and transpiration rate (*Tr*) also increased significantly. However, exogenous nitric oxide application markedly decreased membrane permeability, rate of $O_2^{\cdot-}$ production, the contents of malondialdehyde (MDA) and H_2O_2, and intercellular CO_2 concentration (*Ci*) under 50 mmol/L NaCl stress.

Keywords nitric oxide, NaCl stress, cucumber seedling, active oxygen species, photosynthesis

1 Introduction

Salinity is a major factor limiting plant growth and productivity (Parida and Das, 2005). It was reported that crops on about 2.3×10^9 hm^2 irrigation land are threatened by salinity in the world, accounting for one-third of total irrigation area. There are about 2.001×10^7 hm^2 waste land of salinity and 6.67×10^6 hm^2 plantation of salinity in China, occupying 25% of the total plantation area. Excessive irrigation and lack of rainfall also increase the extent of salinity and the acreage of salinity. Soil salinity also becomes a common problem with the increase of protected cultural area in the world (Wei et al., 2004).

Nitric oxide (NO) is an important redox and toxic molecule in animals and plants, and is also a kind of reactive nitrogen species. NO in plants is mainly produced by NO synthase (NOS, EC 1.14.13.39) and nitrate reductase (NR, EC 1.6.6.1/2) (Yamasaki et al., 1999; Wendehenne et al., 2001). NO has dual roles in plants. Its functions vary with different concentrations, position of action, and cellular physiological conditions. On one hand, NO can directly act with effective molecule or indirectly change cellular redox voltage, and participate in plant growth and signal transduction process of adaptation to the environment. On the oth er hand, interaction of a high concentration of NO and $O_2^{\cdot-}$ produces a great amount of $ONNO^-$, and the combination of $ONNO^-$ with H^+ results in ONNOH damages to the structure and function of biological macromolecules (Zhang and Liu, 2004).

Studies showed that NO exists widely in plants (Ruan et al., 2004), and is involved in respiration (Millar and Day, 1996), photomorphogenic responses (Beligni and Lamattina, 2000), seed germination (Giba et al., 1998), senescence (Leshem et al., 1998), stress responses (Ruan et al., 2004), programmed cell death (Beligni et al., 2002), and disease resistance (Durner et al., 1998; Van Camp et al., 1998; Chandok et al., 2003) in plants, but we know very little about the mechanism of NO in these physiological processes from the present literature (Liu et al., 2004). Researchers found that exogenous NO protects potato plants against cellular damage produced by methylviologen herbicides (Beligni and Lamattina, 1999a). The pretreated rice seedlings with low NO concentration permits the survival of more green leaf tissue and of high quantum yield for photosystem II than in the control, under both salt and heat stresses (Akio et al., 2002). NO can decrease oxidative damage of roots in wheat seedling under salt stress (Chen et al., 2004). NO could increase the activity of plasma member H^+-ATPase caused by NaCl stress,

Translated from *Acta Ecologica Sinica*, 2007, 27(2): 546–553 [译自: 生态学报]

FAN Huaifu, GUO Shirong (✉), JIAO Yansheng, ZHANG Runhua, LI Juan
College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China
E-mail: srgou@njau.edu.cn

which induces the ratio of potassium to sodium in tissues to increase the plant adaptations to salt stress (Zhao et al., 2004). However, we know very little about the effects of NO on cucumber under NaCl stress. There are hardly any reports about the relationship between NO and salt tolerance of cucumber. Therefore, we investigated the effects of NO on alleviating the injury to cucumber seedlings and its relevant mechanism under NaCl stress.

2 Materials and methods

2.1 Materials and treatments

The experiments were carried out in a greenhouse of Nanjing Agricultural University, China. Cucumber (*Cucumis sativus* L. cv. Jinchun 2) seeds were placed on filter paper moistened with distilled water in sterile Petri plates and allowed to germinate in the dark in a thermostatically controlled chamber at 28°C for about 30 h. The germinated seeds were sown in quartz sands. The average day/night temperatures were 25°C–30°C/16°C–20°C under natural light. At the second fully expanded-leaf stage, the cucumber leaves were numbered from apical to basal nodes. Seedlings were taken out of the plastic plates, and the roots were rinsed with distilled water. Some of the selected uniform seedlings were transferred into plastic vessels filled with 20 L Shanqi recipe of cucumber nutrient solution, which was aerated for 40 min per hour, and allowed to continue to grow for additional three days at the same conditions. Then the seedlings were treated with one of the following methods: (1) Shanqi recipe of cucumber nutrient solution; (2) Shanqi recipe of cucumber nutrient solution containing 50 mmol/L NaCl; (3) Shanqi recipe of cucumber nutrient solution containing sodium nitroprusside (SNP) (10, 25, 50, 100, 200 or 400 μmol/L) plus 50 mmol/L NaCl; (4) Shanqi recipe of cucumber nutrient solution containing 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$. SNP was used as NO donor. Because the decomposition of 100 μmol/L SNP generates 1 μmol/L NO_2^- as by-product at most, the treatment of Shanqi recipe of cucumber nutrient solution containing 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$ was carried out as a control.

Cucumber seedling leaves were harvested 0, 2, 4, 6, 8 days after treatment and used for analysis immediately, by measuring the photosynthetic indexes, determining photosynthetic pigment contents and weighing fresh and dry weights after the eight-day treatment. All the solutions were renewed every two days to maintain the identical concentrations. All experiments were performed at least three times.

2.2 Determination of plant height, stem thickness, and fresh and dry weights

Plant height was measured with a ruler; stem thickness (just above the ground) was measured with a vernier caliper. The cucumber seedlings were washed with tap water for 2–3 times, then rinsed twice with distilled water and gently blotted dry with a paper towel and weighed for their fresh weights. Samples were incubated at 105°C for 15 min, then at 75°C until reaching a constant weight, and weighed for dry weight.

2.3 Determination of chlorophyll-a (Chla), chlorophyll-b (Chlb) and carotenoid (Car) concentrations

Chla, Chlb, Car concentrations were assayed according to Yu et al. (2002).

2.4 Determination of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities

The superoxide dismutase (SOD) activity was assayed according to Giannopolitis and Ries (1977); the peroxidase (POD) activity was measured according to Zeng et al. (1997); the catalase (CAT) activity was determined according to Dhindsa et al. (1982); and the ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981).

2.5 Determination of malondialdehyde (MDA) and H_2O_2 contents as well as the rate of $O_2^{\cdot-}$ production

Malondialdehyde content was estimated following the method of Health and Packer (1986). Membrane electrolyte leakage was determined according to Scotti and Thu (1997). Rate of $O_2^{\cdot-}$ production was measured as described by Wang and Luo (1990). H_2O_2 content was estimated as described by Uchida et al. (2002).

2.6 Determination of proline (Pro) content

Pro content was measured as described by Zhao and Liu (2000).

2.7 Determination of photosynthetic characteristics

Pn, *Gs*, *Ci* and *Tr* of the second fully-expended leaf were measured with a Li-6400 photosynthetic measurement system (Li-Cor Ltd., USA). Conditions for measurement: 800 μmol photos/$m^2 \cdot s$, CO_2 from the height of 3–4 m above ground, and 25°C in leaf chamber.

All data presented are the mean values. The measurement was done with three replicates. Statistical assays were carried out by the ANOVA test and the means were compared by the Duncan's multiple range test. The SAS software (SAS Institute, Cary, NC, USA) was used for the analysis of variance (ANOVA). Comparisons with $P < 0.05$ were considered significantly different.

3 Results

3.1 Effect of exogenous NO on the plant growth of seedlings under NaCl stress

Compared with those of control, plant height, stem thickness, fresh weight and dry weight significantly decreased under NaCl stress (Table 1). The growth of seedlings was markedly inhibited by NaCl stress. The inhibition was reduced by adding 10–400 μmol/L exogenous SNP to the solution in a dose-dependent manner. The effects of 50 μmol/L exogenous SNP were significant. Plant height, stem thickness, fresh and dry weights were obviously elevated. Since 100 μmol/L SNP was most effective, we chose this concentration for the succeeding experiments.

Table 1 Effects of different concentrations of exogenous SNP on alleviating the injury to cucumber seedlings under NaCl stress

Treatment	Plant height /cm	Stem thickness /mm	Fresh weight /(g·plant^{-1})	Dry weight /(g·plant^{-1})
NaCl	14.94e	4.77d	19.56e	1.54e
NaCl + 10 μmol/L SNP	18.41d	4.98cd	22.69de	1.90d
NaCl + 25 μmol/L SNP	19.68cd	4.96cd	23.29de	2.00cd
NaCl + 50 μmol/L SNP	20.38bcd	5.13bcd	25.03cd	2.00cd
NaCl + 100 μmol/L SNP	23.00b	5.49ab	31.17b	2.42b
NaCl + 200 μmol/L SNP	22.05bc	5.32bc	27.70bc	2.24bc
NaCl + 400 μmol/L SNP	20.54bcd	5.26bc	26.64cd	2.17bcd
CK	29.03a	5.91a	43.00a	2.80a

Note: The letters in each column denote the significant level at 0.05 (the same below).

3.2 Effect of exogenous NO on the antioxidant system in leaves of seedlings under NaCl stress

As shown in Fig. 1, the SOD, POD, CAT, and APX activities in cucumber leaves under NaCl stress were higher than those of the control from 0 to 8 days of treatment, but 1 μmol/L $NaNO_2$ had no obvious influence on seedlings under NaCl stress. The SOD activity in leaves had a dramatic increase in cucumber leaves when they subjected to salinity, and was the highest after 6-day treatment (Fig. 1(A)). Exogenous NO increased the SOD activity of leaves under NaCl stress, and the SOD activity continued to rise from 0 to 8 days. The difference was the biggest after eight-day treatment under NaCl stress between only NaCl stress and NaCl stress plus exogenous SNP. The SOD activity of leaves with SNP in solution was 149.35% of that in leaves only under NaCl stress and 224.57% of that in leaves of control, respectively.

The POD activity in leaves increased after treatment of NaCl stress and continued to rise. The POD activity with SNP in solution was higher than that without SNP. The POD

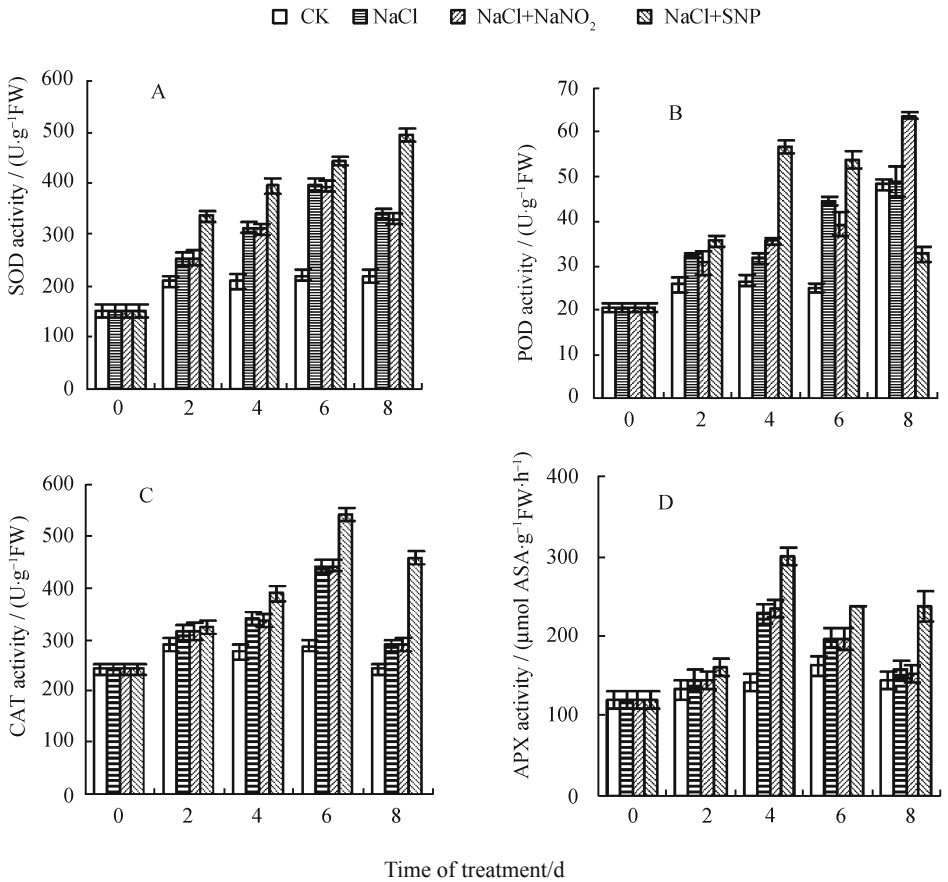

Fig. 1 Effects of exogenous NO on (A) SOD activity, (B) POD activity, (C) CAT activity, (D) APX activity in leaves of cucumber seedlings under NaCl stress

activity in leaves under NaCl stress with SNP in solution and without SNP had significant difference on the fourth, sixth, and eighth days (Fig. 1(B)).

The CAT activity in leaves increased slowly in early days under NaCl stress and increased quickly after 4 days of treatment. The activity was the highest on the sixth day. Compared with that without SNP, the CAT activity in leaves under NaCl stress with SNP in solution increased obviously on the second day after treatment. The CAT activity in leaves under NaCl stress with SNP in solution was 157.90% of that in leaves only under NaCl stress and 188.35% of that in leaves of control after eight days of treatment, respectively (Fig. 1(C)), but had statistical significance.

Similarly, the APX activity in leaves also increased under NaCl stress and was the highest on the sixth day after treatment. The APX activity in leaves under NaCl stress with SNP in solution had obvious increase on the second day and was also higher than that without SNP under NaCl stress after treatment, and the difference between the two treatments was significant (Fig. 1(D)).

3.3 Effects of exogenous NO on membrane permeability, MDA and H_2O_2 contents as well as the rate of $O_2^{\cdot-}$ production in leaves under NaCl stress

Membrane permeability in leaves increased under NaCl stress. Plenty of electrolytes leaked out. Membrane permeability improved rapidly after four days of treatment, and increased slowly then. Membrane permeability in leaves under NaCl stress with SNP in solution at the sixth and eightth days of treatment were 153% and 200% of that in leaves of control, respectively. For the treatment of NaCl stress without SNP in solution, in six-day treatment and eight-day treatment, the permeabilities were 200% and 231% of that in leaves of control, respectively, after treatment. Membrane permeability in leaves under NaCl stress with SNP in solution and without SNP was significantly different (Fig. 2(a)). All of the results showed that exogenous NO decreased the membrane permeability in leaves of cucumber seedlings and reduced the electrolyte leakage, so as to maintain the structural integrity of cells.

Rate of $O_2^{\cdot-}$ production in leaves under NaCl stress increased rapidly, and was the highest at the sixth day after treatment. Rate of $O_2^{\cdot-}$ production under NaCl stress decreased obviously as a result of adding exogenous SNP to solution. Rate of $O_2^{\cdot-}$ production under NaCl stress with SNP in solution had the same rate with that of control eight days after treatment, but rate of $O_2^{\cdot-}$ production in leaves under NaCl stress without SNP in solution was obviously higher than that of control (Fig. 2(b)).

The MDA content continued to rise during the whole time of treatment under NaCl stress, which suggested cells had been damaged. Solution plus SNP alleviated the

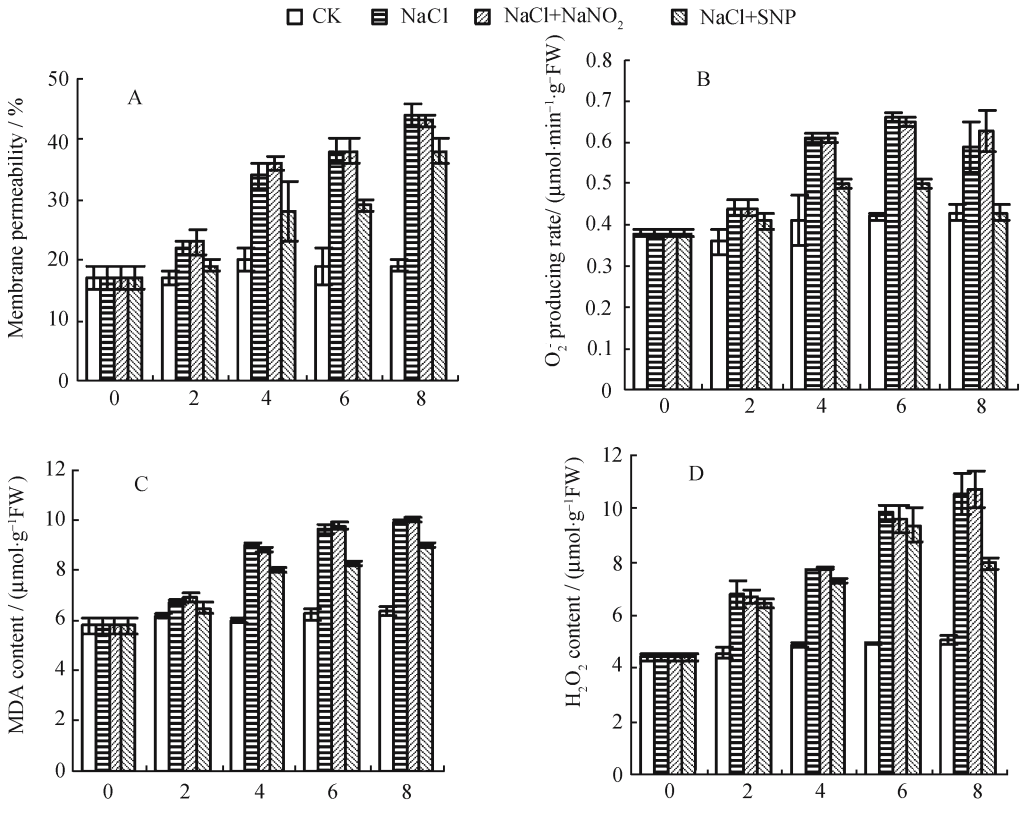

Fig. 2 Effects of exogenous NO on (A) membrane permeability, (B) $O_2^{\cdot-}$ producing rate, (C) MDA content, (D) H_2O_2 content in leaves of cucumber seedlings under NaCl stress

accumulation of MDA in leaves (Fig. 2(C)). NO treatments reduced lipid peroxidation.

Compared with that of control, H_2O_2 content in leaves under NaCl stress increased obviously, and increased slowly in the early stage and then rapidly after four days of treatment. SNP reduced the accumulation of H_2O_2 in leaves under NaCl stress. H_2O_2 content in leaves under NaCl stress with SNP in solution and without SNP was 1.58 and 2.09 times of that in leaves of control eight days after treatment, respectively (Fig. 2(D)).

The 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$ in solution had no obvious effects on membrane permeability, MDA and H_2O_2 contents as well as the rate of $O_2^{\cdot-}$ production in leaves of cucumber seedlings.

3.4 Effects of exogenous NO on Pro content in leaves of cucumber under NaCl stress

Pro is an important osmotic adjustable substance and antioxidant. Pro content in leaves under NaCl stress significantly increased two days after treatment and continued to rise in the next treatment. The Pro content in leaves under NaCl stress was higher than that in leaves growing under normal conditions. SNP increased the Pro content under NaCl stress. The Pro content with SNP under NaCl stress was 112% of that without SNP under NaCl stress after eight days after treatment with significant difference (Fig. 3). The Pro content under normal conditions was always at a low level. The 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$ in solution had no obvious effects on Pro content.

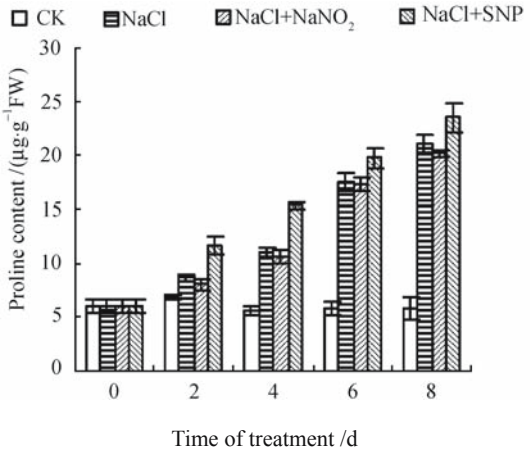

Fig. 3 Effects of exogenous NO on Pro content in leaves of cucumber seedlings under NaCl stress

3.5 Effects of exogenous NO on photosynthetic pigment contents and photosynthetic indexes in leaves of cucumber under NaCl stress

The contents of Chla, Chlb, Car, Chla + Chlb in leaves under NaCl stress were lower than those of the control after eight days of treatment (Table 2). Exogenous NO increased the photosynthetic pigment contents in leaves under NaCl stress, and had obvious difference with those under NaCl stress and control. The 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$ in solution had no obvious effects on these photosynthetic pigments.

Table 2 Effects of exogenous nitric oxide on photosynthetic pigment contents in leaves of cucumber seedlings under NaCl stress

Treatment	Chla /(mg·g^{-1}FW)	Chlb /(mg·g^{-1}FW)	Car /(mg·g^{-1}FW)	Chla + Chlb /(mg·g^{-1}FW)
NaCl	1.12 ± 0.02c	0.34 ± 0.02c	0.86 ± 0.04c	1.46 ± 0.04c
NaCl + $NaNO_2$	1.19 ± 0.08c	0.37 ± 0.02bc	0.89 ± 0.06c	1.56 ± 0.10c
SNP + NaCl	1.56 ± 0.04a	0.49 ± 0.02a	1.19 ± 0.02a	2.05 ± 0.05a
CK	1.35 ± 0.02b	0.42 ± 0.01b	1.03 ± 0.01b	1.77 ± 0.02b

Pn, *Gs* and *Tr* values in leaves under NaCl stress were lower than those of the control after eight days of treatment, but *Ci* was higher (Table 3). Compared with that without SNP, *Pn* in leaves under NaCl stress with SNP in solution increased with *Ci* decreasing. The 50 mmol/L NaCl plus 1 μmol/L $NaNO_2$ in solution had no obvious effects on these photosynthetic indexes.

Table 3 Effects of exogenous nitric oxide on photosynthetic indexes in leaves of cucumber seedlings under NaCl stress

Treatment	Net *Pn* /(μmol·m^{-2}·s^{-1})	*Gs* /(μmol·m^{-2}·s^{-1})	*Ci* /(μL·L^{-1})	*Tr* /(μmol·m^{-2}·s^{-1})
NaCl	5.81 ± 0.18c	0.16 ± 0.02c	311.67 ± 8.21a	1.67 ± 0.13c
NaCl + $NaNO_2$	6.05 ± 0.43c	0.17 ± 0.03c	313.33 ± 18.37a	1.72 ± 0.19c
SNP + NaCl	10.87 ± 0.72b	0.27 ± 0.02b	288.67 ± 10.89b	2.50 ± 0.16b
CK	15.20 ± 0.12a	0.59 ± 0.01a	268.00 ± 10.58c	3.73 ± 0.01a

4 Discussion

Salt stress lead to injury to plants in many aspects. It damages the balance of nutrition in plants, restricts the absorption and transport of nutritive elements, changes the concentration and types of ions in plant cells, and results in damaging the integrity of the membrane and decreasing the functions of enzymes. The membrane system, especially for cell membranes, is the initial part being damaged and the most sensitive section under NaCl stress (Liu et al., 2005). The theory of free radicals states that salt stress firstly results in the imbalance of ions and osmotic stress, such as accumulation of many kinds of reactive oxygen species, breakage of membrane, degradation of chlorophyll, denaturalization of protein, rupture of nucleic acid and even cell death (Zhu, 2002).

NO is a kind of normal metabolite or by-product, and also an important messenger molecule. Its physiological effects include adjustment of reactive oxygen species and signal transduction (Delledonne et al., 1998; Garcia-Mata and Lamattina, 2001). The high level of reactive oxygen species may induce membrane lipid peroxidation, finally resulting in

the breakage of membrane integrity. Our studies suggested that 100 μmol/L exogenous SNP could significantly alleviate the inhibition to growth of cucumber seedlings subjected to 50 mmol/L NaCl stress, and markedly improved the plant height, stem thickness, and fresh and dry weights. It was related to the direct effects of NO on the components of cell wall by apoplast. NO relaxed the cell wall, acted on the phospholipids bilayer, improved the fluidness of the membrane, cell enlargement and plant growth (Leshem and Hamaraty, 1996). SNP improved the activity of antioxidant enzymes of cucumber seedling leaves under NaCl stress to a different extent, and reduced the rate of $O_2^{\cdot-}$ production, membrane permeability, H_2O_2 and MDA contents simultaneously, but NO_2^-, as the major by-product of SNP, had no such effects. SOD was an induced enzyme, and could be induced by substrates. Salt stress accelerated the $O_2^{\cdot-}$ production and induced a great increase of the SOD activity. Solution plus SNP under NaCl stress improved the SOD activity in leaves, which demonstrated that SNP alleviated the injury to cucumber seedlings under NaCl stress. NO disproportioned $O_2^{\cdot-}$ to H_2O_2, so the content of H_2O_2 increased with the improved SOD activity. Under NaCl stress, some enzymes eliminated and decompounded H_2O_2, such as POD, CAT, APX, and so on, and their activity increased slowly and in a small range, increased in a short period of time and subsequently decreased. Exogenous SNP could markedly improve the SOD, POD, CAT, APX activities, thereby improving the ability of scavenging free radicals and alleviating the injury, and decreasing membrane permeability and MDA content. There was another possibility that NO directly reacted with $O_2^{\cdot-}$ (Beligni and Lamattina, 1999b; Beligni et al., 2002). NO could improve the activity of protective enzymes; the main reason was that NO had an intense affinity to the enzymes containing iron. For example, NO could participate in a series of resistant physiological reaction by adjusting activities of CAT, APX, COX and other relative enzymes containing hemachrome iron or by restraining activity of aconitase without hemachrome iron (Wang et al., 2004). The increase of SOD, POD, CAT, APX activities reduced much production of reactive oxygen species, such as $O_2^{\cdot-}$, H_2O_2 and so on, which makes it possible to increase osmotic adjustment ability and salt tolerance (Zhu, 2002). Exogenous NO reduced the membrane permeability and membrane lipid peroxidation, and prevented the electrolyte leakage, which suggested exogenous NO possessed the functions of repairing and protecting the cell membrane to alleviate the injury in the cell membrane system.

Pro not only was an osmotic adjustment substance, but also might eliminate reactive oxygen species and improve the antioxidant ability, stabilize the structure of biological macromolecules, decrease cell acidity and relieve the toxicity of NH_4^+ (Hou and Tang, 1999). Exogenous NO could promote the accumulation of Pro in annual ryegrass under chilling stress, and enhanced the chilling resistance (Ma et al., 2005). Ruan et al. (2001) reported that exogenous NO might enhance the salt resistance of wheat seedling by improving the Pro content in leaves. Our experimental results indicated that exogenous NO could improve the Pro content in leaves of cucumber seedlings. Therefore, exogenous NO might enhance the salt tolerance of cucumber seedlings by improving the accumulation of Pro.

Jiang et al. (1994) proved that degradation of chlorophyll was induced mainly by the reactive oxygen species, but the increase of leakage of electrolyte was positively correlated to membrane lipid peroxidation. Exogenous NO increased the chlorophyll contents and activities of antioxidant enzymes, reduced the electrolyte leakage, and consequently alleviated the injury to cucumber seedlings due to NaCl stress. Increase of chlorophyll contents might be related to some active synthesizing enzymes in biosynthesis by NO.

Under salt stress, plant photosynthesis is affected significantly (Parida and Das, 2005). Maintaining photosynthesis is an important mechanism for plants to adapt to salt tolerance (Liu and Wang, 1998). In our study, *Pn* and *Gs* decreased markedly, while *Ci* increased obviously. The main reason for the decline of net *Pn* was mostly due to decline of activity of photosynthesis in cells of leaves. Exogenous SNP reduced the decrease of photosynthesis in cells of leaves because of salt stress, and improved chlorophyll content and *Pn*, *Tr* and *Gs*, but decreased *Ci*. Higher *Gs* values showed higher transduction ability of the substrate in relation to net *Pn*, which supplied the basis of physiology for assimilating more photosynthetic production. Increase of *Tr* enhanced the power of absorption and transportation for water, which was beneficial to the increase of photosynthesis and salt tolerance.

Acknowledgements This research was funded by the Three Engineering Projects of Agriculture of Jiangsu Province (No. SX(2005)088) and the Scientific Research Foundation of Ph. D. Programs, China (No. 2005307031).

References

Akio U, Andre T J, Takashi H, Teruhiro T, Tetsuko T (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci, 163: 515–523

Beligni M V, Fath A, Bethake P C, Lamattina L, Jones R (2002). Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol, 129: 1642–1650

Beligni M V, Lamattina L (1999a). Nitric oxide protects against cellular damage produced by methylviologen herbicides in potato plants. Nitric Oxide, 3(3): 199–208

Beligni M V, Lamattina L (1999b). Is nitric oxide toxic or protective? Trends Plant Sci, 4(8): 299–300

Beligni M V, Lamattina L (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta, 210: 215–221

Chandok M R, Ytterberg A J, Van Wijk K J, Klessing D F (2003). The pathogen-inducible nitric oxide synthase (iNOS) in plant is avariant of the P protein of the glycine decarboxylase complex. Cell, 113(4): 469–482

Chen M, Shen W B, Ruan H H, Xu L L (2004). Effects of nitric oxide on root growth and its oxidative damage in wheat seedling under salt stress. Journal of Plant Physiology and Molecular Biology, 30(5): 569–576 (in Chinese)

Delledonne M, Xia Y J, Dioxin R A (1998). Nitric oxide function as a signal in plant disease resistance. Nature, 394(6693): 585–588

Dhindsa R S, Plumb-Dhindsa P, Thorpe T A (1982). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased leavels dismutase and catalase. J Exp Bot, 32: 91–101

Durner J, Wendehenne D, Klessing D F (1998). Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA, 95: 10328–10333

Garcia-Mata C, Lamattina L (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant response against drought stress. Plant Physiol, 126: 1196–1204

Giannopolitis C N, Ries S K (1977). Purification and quantitative relationship with water-soluble protein in seedling. Plant Physiol, 59: 315–318

Giba Z, Grubisic D, Todorovic S, Sajc L, Stojakovic D, Konjevic R (1998). Effect of nitric oxide releasing compounds on phytochrome-controlled germination of empress tree seeds. Plant Growth Regul, 26: 175–181

Health R L, Packer L (1986). Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys, 125: 189–198

Hou C X, Tang Z C (1999). Function and mechanism of compatible solutes. Plant Physiology Communications, 35(1): 1–7 (in Chinese)

Jiang M Y, Yang W Y, Xu J, Chen Q Y (1994). Active oxygen damage effect of chlorophyll degradation in rice seedlings under osmotic stress. Acta Bot Sin, 36(4): 289–295

Leshem Y Y, Hamaraty E (1996). Plant aging: the emission of NO and ethylene and effect of NO-releasing compounds on growth of pea (*Pisum sativum* L.) foliage. J Plant Physiol, 148: 258–263

Leshem Y Y, Wills R B H, Ku V V V (1998). Evidence for the function of the free radical gas-nitric oxide (NO) as an endogenous maturation and senescence regulating factor in higher plant. Plant Physiol Biochem, 36: 825–833

Liu K L, Han H R, Xu Y J, Ling T F, Liu Z B, Sun Y G, Hua R, Shen W B (2005). Exogenous nitric oxide alleviates salt stress-induced membrane lipid peroxidation in rice seedling roots. Chinese J Rice Sci, 19(4): 333–337 (in Chinese)

Liu P C, Wang H, Cheng J Q, Huang J CH (2004). Regulation of nitric oxide on drought-induced membrane lipid peroxidation in wheat leaves. Acta Bot Boreal Occident Sin, 24(1): 141–145 (in Chinese)

Liu Y L, Wang L J (1998). Responses to salt stress in plants and its salt tolerance. In: Yu S W, Tang Z C, eds. Plant Physiology and Molecular Biology. Beijing: Science Press, 752–769 (in Chinese)

Ma X L, Wei X H, Long R J, Cui W J, Wan Y L (2005). Studies on mechanism of enhancing the chilling resistance of annual ryegrass by exogenous nitric oxide. Acta Ecologica Sinica, 25(6): 1269–1274 (in Chinese)

Millar A H, Day D A (1996). Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. FEBS, 398: 155–158

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorabate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 22: 867–880

Parida A K, Das A B (2005). Salt tolerance and salinity effects on plants: A review. Ecotoxicology and Environmental Safety, 60: 324–349

Ruan H H, Shen W B, Xu L L (2004). Nitric oxide modulates the activities of plasma membrane ATPase and PPase in wheat seeding roots and promotes the salt tolerance against salt stress. Acta Bot Sin, 46(4): 415–422 (in Chinese)

Ruan H H, Shen W B, Ye M B, Xu L L (2001). Protective effects of nitric oxide on salt stress-induced oxidative damage to wheat (*Trriticum aestivum* L.) leaves. Chin Sci Bull, 46(23): 1993–1997 (in Chinese)

Scotti C, Thu P T (1997). Effect of abscisic pretreatment on membrane leakage and lipid composition of *Vigna unguiculata* leaf discs subjected to osmotic stress. Plant Sci, 130: 11–18

Uchida A, Andre T J, Takashi H (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci, 163: 515–523

Van Camp W, Van Montagu M, Inze D (1998). H_2O_2 and NO: Redox signals in disease resistance. Trends Plant Sci, 3: 330–334

Wang A G, Luo G H (1990). Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plant. Plant Physiol Commun, 26(6): 55–57 (in Chinese)

Wang X Y, Shen W B, Xu L L (2004). Exogenous nitric oxide alleviates osmotic stress-induced membrane lipid peroxidation in wheat seedling leaves. Journal of Plant Physiology and Molecular Biology, 30(2): 195–200 (in Chinese)

Wei G Q, Zhu Z J, Fang X Z, Li J, Cheng J Q (2004). The effects of NaCl stress on growth, chlorophyll fluorescence characteristics and active oxygen metabolism in seedlings of two cucumber cultivars. Scientia Agricultura Sinica, 37(11): 1754–1759 (in Chinese)

Wendehenne D, Pugin A, Klessig D F (2001). Nitric oxide: Comparative synthesis and signaling in animal and plant cells. Trend Plant Sci, 6(4): 177–183

Yamasaki H, Shaniohi S Y, Takahashi S (1999). An alternative pathway for nitric oxide production in plant: new feature of an old enzyme. Trends Plant Sci, 6(4): 177–183

Yu B J, Li S N, Liu Y L (2002). Comparision of ion effects of salt injury in soybean seedlings. Journal of Nanjing Agricultural University, 25(1): 5–9 (in Chinese)

Zeng S X, Wang Y R, Li M R (1997). Comparison of changes of membrane protective system in rice seedlings during enhancement of chilling resistance by different stress pretreatment. Acta Botanica Sinica, 39(4): 308–314 (in Chinese)

Zhang Y Y, Liu Y L (2004). Source and function of nitric oxide in plants. Acta Bot Boreal Occident Sin, 24(5): 921–929

Zhao F G, Liu Y L (2000). The biosynthesis of polyamines is more sensitive than that of proline to salt stress in barley seedlings. Acta Phytophysiolgica Sin, 26(4): 243–349 (in Chinese)

Zhao L Q, Zhang F, Guo J K, Yang Y L, Li B B, Zhang L X (2004). Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. Plant Physiol, 134: 849–857

Zhu J K (2002). Salt and drought stress signal transduction in plants. Annu Rev Plant Physiol Mol Biol, 53: 247–273