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Nitric Oxide Involved in the Abscisic Acid Induced Proline Accumulation in Wheat Seedling Leaves Under Salt Stress

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Abstract: Exogenous nitric oxide (NO) releaser sodium nitroprusside (SNP) with different concentrations from 0.01 to 5.00 mmol/L induced proline accumulation in wheat (*Triticum aestivum* L. cv. Yangmai 158) seedling leaves under 150 mmol/L salt stress in a dose-dependent manner. It was most effective at 0.1 mmol/L SNP, and the combination treatments with two NO scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) and hemoglobin, separately reverted the 0.1 mmol/L SNP induced proline accumulation. Meanwhile, the proline accumulation induced by NO might be of benefit to the water retention in wheat seedling leaves when subjected to salinity, and exogenous 0.1 mmol/L SNP treatment also dramatically activated the synthesis of endogenous abscisic acid (ABA), and the employment of hemoglobin further indicated that NO might be downstream of the ABA induced proline accumulation in wheat seedling leaves under 150 mmol/L salt stress, but there did not exist synergism between NO and ABA signaling toward proline accumulation. Detection of proline synthesis and degradation demonstrated that exogenous NO induced proline accumulation in a phase dependent manner, mainly by enhancing the activities of Δ^1-pyrroline-5-carboxylate synthetase (P5CS) within the first 4 d of treatment and inhibiting activities of proline dehydrogenase (ProDH) 4 d later up to 8 d. And ABA showed a weak effect on P5CS and ProDH activities in comparison with NO treatment. Additionally, Ca^{2+} was confirmed as the important intermediates during the NO signaling pathway in proline accumulation under salinity conditions.

Key words: wheat (*Triticum aestivum*); nitric oxide (NO); abscisic acid (ABA); proline; salt stress

Salt stress is a key factor that reduces crop production in agriculture (Zhu, 2002). Plants produce various biochemical and physiological responses to survive this adverse environmental condition, such as the accumulation of proline, glycine betaine and sugar alcohols (Delauney and Verma, 1993). In fact, free proline accumulates in a wide variety of higher plants, such as tobacco, soybean, barley, wheat and rice (Yoshiba *et al.*, 1997). Proline may function as an osmoticum, a hydroxy-radical scavenger, a compatible solute that protects enzymes and a sink of energy and reducing power (Verbruggen *et al.*, 1993). Until now, the metabolism of proline in plants had also been basically reviewed (Hare *et al.*, 1999). For example, proline accumulation in response to salt stress in plants is mainly mediated by two enzymes, Δ^1-pyrroline-5-carboxylate synthetase (P5CS), a rate-limiting enzyme in proline biosynthesis, and proline dehydrogenase (ProDH), a mitochondrial enzyme involved in the first step of the conversion of proline to glutamic acid (Chen *et al.*, 2001).

Nitric oxide (NO) is a short life bioactive molecule in plants, convincing data have been obtained by the NO-induced increase in plant tolerance or resistance against various abiotic and biotic stresses, such as pathogen disease (Delledonne *et al.*, 1998), drought (García-Mata and Lamattina, 2001; Zhang *et al.*, 2003), salinity (Ruan *et al.*, 2002; 2004; Uchida *et al.*, 2002), UV-B irradiation (Mackerness *et al.*, 2001) and wounding (Huang *et al.*, 2004). While abscisic acid (ABA) had been known playing important roles in the tolerance of plants to high salinity, there also exists both ABA-dependent and ABA-independent signal transduction cascades between the initial signal of salt stress and the expression of specific tolerance genes (Shinozaki and Shinozaki, 1997). Meanwhile, it has been suggested that NO exists net cross talk with ABA, cADPR, cGMP, ion channels, Ca^{2+}, and others in plants (García-Mata and Lamattina, 2002; Neill *et al.*, 2002; Correa-Aragunde *et al.*, 2004). Our previous results (Ruan *et al.*, 2002) had preliminarily proved that NO promoted the accumulation of proline, which might participate the protective effect of NO on salt-induced oxidative damage to wheat seedlings. But how NO regulates the proline accumulation and its role in ABA pathway underlying proline accumulation under salt stress have not been well documented. In the present study, we demonstrated the relationship of NO and ABA in the process of proline accumulation responding to salt stress, and the effects of NO on the limiting

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enzymes controlling proline metabolism and the intermediates involving in NO signaling were also investigated.

1 Materials and Methods

1.1 Plant materials and treatments

Sterilized wheat seeds (*Triticum aestivum* L. cv. Yangmai 158, kindly supplied by Jiangsu Agricultural Institutes, Jiangsu Province, China) with 0.1% $HgCl_2$, were cleaned in distilled water and germinated at 25 ℃ in the dark. Selected the identical buds and grown hydroponically in chamber (12-h light period, 25 ℃, humidity 60%; 12-h dark period, 18 ℃, humidity 60%) with roots submerged in Hoagland solution under irradiance of 100 μmol·m^{-2}·s^{-1} provided by fluorescent lamps. The solutions were renewed every day until the second fully expanded leaves appeared.

SNP ($(Na_2Fe(CN)_5)$ NO, Merck, Darmstadt, Germany) was used as NO releaser or donor. 2-(4-carboxyphenyl)-4, 4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO, Sigma, USA) was used as a specific scavenger of NO. Hemoglobin (another NO scavenger) and $LaCl_3$ (a blocker of Ca^{2+} channel) were purchased from Shanghai Boao Ltd., China. ABA was purchased from Sigma (USA). NaCl was directly added to Hoagland solutions, and all above chemicals were added to Hoagland solutions with or without NaCl. Toward the treatments with sodium nitroprusside (SNP), the solutions were sealed to avoid the NO gas letting out the air. Moreover, the treatment solutions were changed every day to maintain the identical concentrations. Finally, the first leaf of wheat seedlings with two fully expanded leaves was cut for further investigation after suitable treatment period.

One g of fresh wheat leaves was dried at 70 ℃ for at least 72 h to determine the dry weight (DW). The following measurements were expressed as the unit of per g DW instead that of per g FW.

1.2 Determination of proline content

Proline was extracted and its concentration was determined by the method of Bates *et al*. (1973). Leaf segments were homogenized with 3% sulfosalicylic acid (W/V) and the homogenate was centrifuged at 3 000*g* for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Contents of proline are expressed as μg /g DW.

1.3 Measurement of relative water content (RWC)

RWC was determined by the method of García-Mata and Lamattina (2001).

1.4 Quantification of ABA

ABA was extracted from leaves with 80% methanol (V/V) and was quantified by the method of HPLC as described by Láng *et al*. (1994).

1.5 Assay of P5CS activity

Activities of P5CS were assayed according to Zhao and Liu (2000). One unit (U) of its activity was defined as 0.5 DA_{435}·g^{-1} DW ·h^{-1}.

1.6 Extraction and active staining of ProDH activity

Extraction and active staining of ProDH activities were performed as described by Zhao and Liu (2000) and Rayapati and Stewart (1991) with some modifications. The supernatant was the crude enzyme extract. Also the protein in the supernatant was determined and the equivalent amounts of protein was loaded during following electrophoresis.

ProDH isozyme was separated by polyacrylamide gel electrophoresis (PAGE, T=7.5 %). The ProDH activity was visualized with staining solutions containing 0.1 mol/L Na_2CO_3-$NaHCO_3$ (pH 10.3), 8 mmol/L proline and 72 μmol/L 2, 6-dichlorphenolindophenol natriumsalz dihydrat. The gels were bathed at 30 ℃ for 5 min with staining solution. Then final concentration of 0.72 mg/mL PMS was added to it until a clear pink band appeared. Discarded the solution and cleaned the gel with distilled water three times to get rid of background. The relative levels of ProDH activities were scanned with dual wavelength TLC scanner (CS-930) at 600 nm, and the data were expressed with its proportion to that of CK, which was standardized as 100%.

1.7 Protein determination

Protein was determined by the method of Bradford (1976) with BSA as the standard.

2 Results

2.1 Effect of NO on the content of proline in wheat seedling leaves under salt stress

In previous results (Ruan *et al.*, 2002), we investigated the protective effects of SNP on wheat seedling leaves under 150 and 300 mmol/L NaCl salt stress, respectively. Here, effects of various SNP ranging from 0.01 to 5 mmol/L on proline accumulation in wheat seedling leaves under 150 mmol/L NaCl salt stress were surveyed. As shown in Fig.1A, 0.01, 0.10 and 1.00 mmol/L SNP treatment all elevated the contents of proline in varying levels through 8 d of treatments, and 0.10 mmol/L SNP showed the maximum induction to proline accumulation compared with control (CK, $P < 0.01$). Contrary to these, 5 mmol/L SNP treatment had no obvious effect on the proline content during these period (Fig.1A). Therefore, it could be summarized that the increase of proline content by SNP was in a dose dependent manner. However, the proline in wheat seedling roots was undetectable absolutely (data not shown).

To be sure that the NO releaser SNP induced proline accumulation in wheat seedling leaves under 150 mmol/L

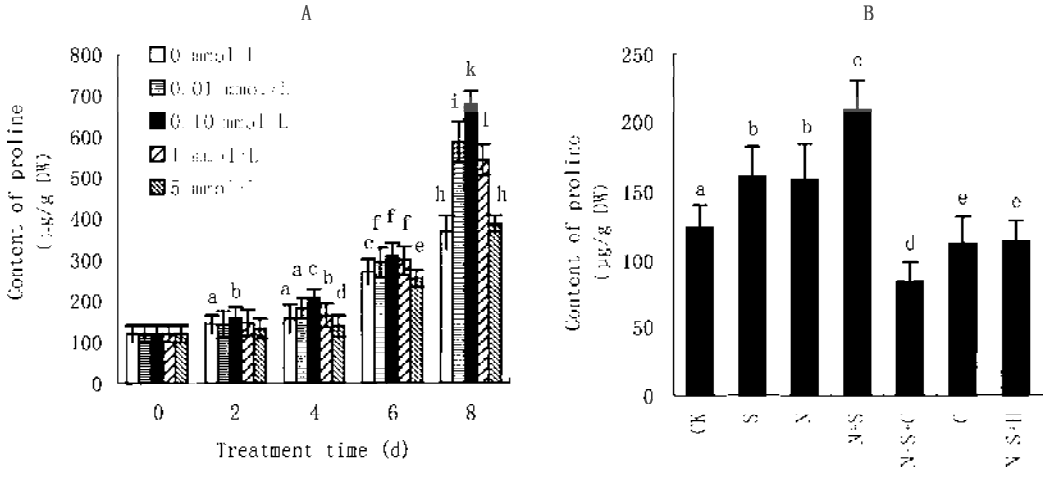

Fig.1. **A.** Effects of increasing concentrations (0, 0.01, 0.10, 1 or 5 mmol/L) of SNP on the content of proline in wheat seedling leaves under 150 mmol/L NaCl salt stress. **B.** Changes of proline content in wheat seedling leaves after 4 d treated with: Hoagland solution (control, CK), CK+0.1 mmol/L SNP (S), CK+150 mmol/L NaCl (N), CK+150 mmol/L NaCl+ 0.1 mmol/L SNP (N+S), CK+150 mmol/L NaCl+0.1 mmol/L SNP +0.2 mmol/L c-PTIO (N+S+C), CK+0.2 mmol/L c-PTIO (C), CK+150 mmol/L NaCl+0.1 mmol/L SNP + 0.1% (W/V) hemoglobin (N+S+H). Bars represent the mean±*SE* of three independent experiments. One way ANOVA was used for comparison between the means. Bars with different letters are significantly different at $P < 0.05$ or $P < 0.01$.

NaCl salt stress was specific to NO, the NO scavenger c-PTIO and hemoglobin (Takahashi and Yamasaki, 2002) were employed. Figure 1B shows that combination with 0.2 mmol/L c-PTIO and 0.1 % hemoglobin (W/V) both dramatically inhibited the 0.1 mmol/L SNP induced proline accumulation by 59.8% and 46.0%, respectively, under salt stress for 4 d. These further confirmed the specific role of NO on the proline accumulation in wheat seedling leaves under salt stress.

Known to all, plant growth responding to salt stress follows a combined effect of osmotic stress and ionic stress. With that purpose, we determined the effects of NO donor SNP on RWC of wheat seedling leaves subjected to 150 mmol/L NaCl salt stress, and found that exogenous 0.1 mmol/L SNP treatment obviously elevated the RWC under salt stress. For example, the RWC in 0.1 mmol/L SNP treated leaves after 2 d was approximately 82.5%, which was statistically significant with that of 73.6 % in non-treated leaves ($P < 0.05$, Fig.2A). Also, the correlation between the percentages of RWC elevation and proline accumulation induced by 0.1 mmol/L SNP under salt stress was analyzed from the result of Fig.2B. The proline accumulation and sustentation of water in wheat seedling leaves induced by NO showed significant positive correlation ($Y = 0.410\ 1\ X + 5.363\ 8$, $r = 0.914\ 7$, $r_{0.01} = 0.878$, Student *t*-test). These suggested that the NO induced proline accumulation might be of benefit to the water retention in seedling leaves when subjected to salinity, which also partially contributed to the promotion of salt tolerance exerted by NO (Ruan *et al.*, 2004).

2.2 Effect of NO in the process of ABA induced proline accumulation in wheat seedling leaves under salt stress

ABA is an important plant growth regulator in the process of plants responding to salinity. We further detected the effect of exogenous NO on the content of endogenous ABA in wheat seedling leaves under 150 mmol/L NaCl salt stress. As shown in Fig.3A, the content of endogenous ABA was primarily elevated under NaCl salt stress alone within the first 4 d of treatment, which might be due to an activation of self-defence in plants responding to severe environment (Campalans *et al.*, 1999), but it gradually decreased after that period time. Compared with 150 mmol/L NaCl stress alone, the content of ABA was further strikingly enhanced by 0.1 mmol/L SNP treatment under salt stress through the first 2 d of treatment ($P<0.05$) and thus maintained in an approximately equivalent level at the following lag phase. Moreover, it was noticeable that 0.1% hemoglobin treatment arrested these effects. These further confirmed that NO could activate the synthesis of ABA, which plays an important role in plant adaptation to salinity.

It has been reported that ABA could enhance the amount of proline in plants (Savouré *et al.*, 1997; Yoshiba *et al.*, 1999). Therefore, effect of NO on the ABA induced proline accumulation under salt stress was further investigated. As shown in Fig.3B, the level of proline was significantly elevated by exogenous supplied ABA in wheat seedling

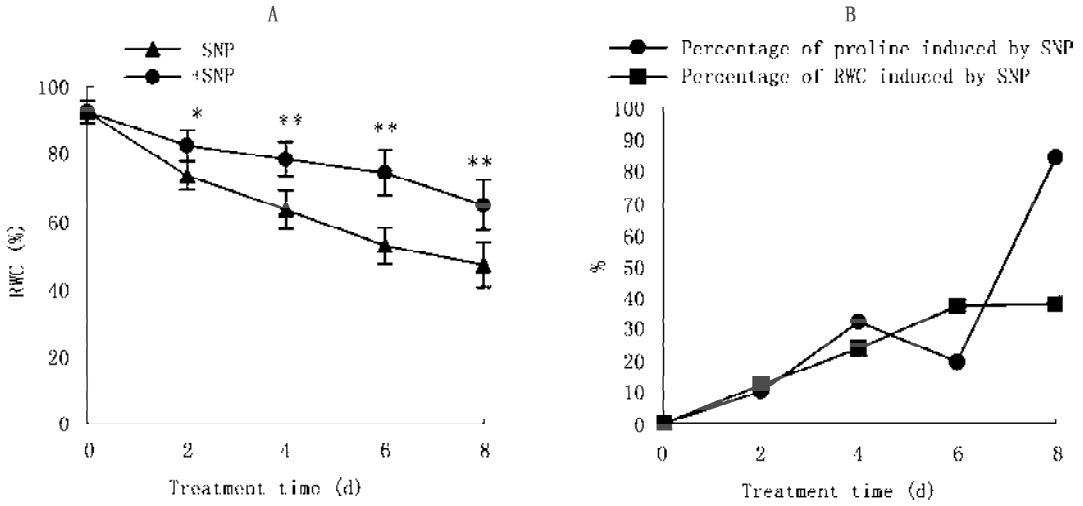

Fig.2. **A.** Time-dependent changes in relative water content (RWC) of wheat seedling leaves subjected to 150 mmol/L NaCl salt stress. + sodium nitroprusside(SNP) (–SNP) represent treatments of Hoagland solution plus 150 mmol/L NaCl with or without 0.1 mmol/L SNP. Each value is the mean ± *SE* of three replicates. One way ANOVA was used for comparisons between the means. The data marked with * and ** are different at a level of significance of $P < 0.05$ and $P < 0.01$, respectively. **B.** Changes of the percentage of RWC elevation and proline induction by 0.1 mmol/L SNP under 150 mmol/L NaCl salt stress.

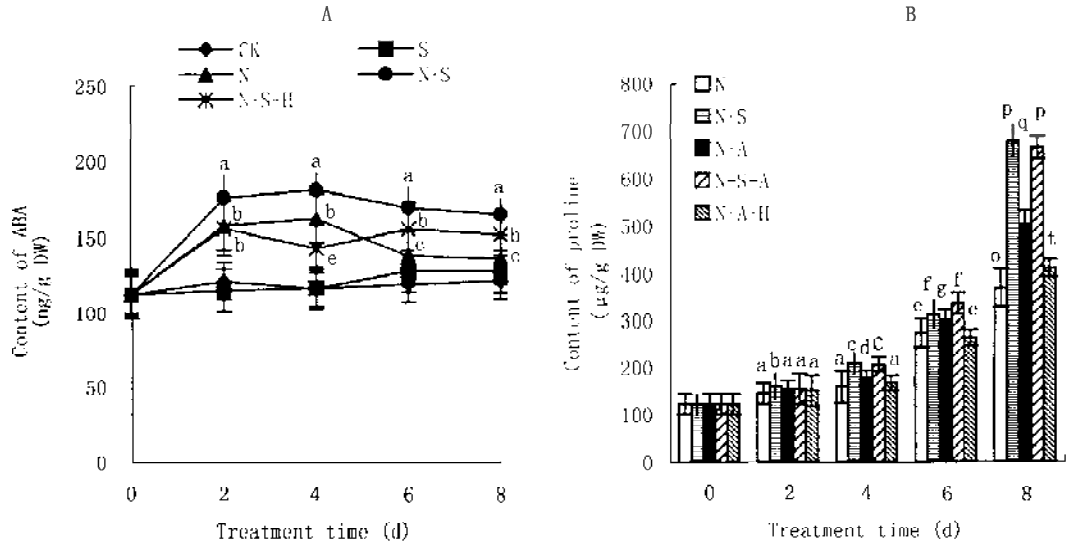

Fig.3. **A.** Exogenous NO induced the synthesis of endogenous ABA in wheat seedling leaves under salt stress. **B.** Effect of NO in ABA signaling pathway inducing proline accumulation in wheat seedling leaves under salt stress. Different treatment represents: Hoagland solution (control, CK), CK+0.1 mmol/L sodium nitroprusside (SNP) (S), CK+150 mmol/L NaCl (N), CK+150 mmol/L NaCl+0.1 mmol/L SNP (N+S), CK+150 mmol/L NaCl+0.1 mmol/L SNP+ 0.1% hemoglobin (N+S+H), CK+150 mmol/L NaCl+0.1 mmol/L SNP+5 μmol/L ABA (N+S+A), CK+150 mmol/L NaCl+5 μmol/L ABA (N+A), CK+150 mmol/L NaCl+5 μmol/L ABA+ 0.1% (W/V) hemoglobin (N+A+H). Bars represent the mean ± *SE* of three independent experiments. One way ANOVA was used for comparisons between the means. Bars with different letters are significantly different at $P < 0.05$ or $P < 0.01$.

leaves under 150 mmol/L NaCl salt stress after 4 d of treatment ($P < 0.05$). However, NO induced the proline accumulation after 2 d of incubation, and the percentages of induction were significantly higher than those of ABA treatment. When combination with 0.1% (W/V) hemoglobin treatment, the ABA induced proline accumulation was obviously fallen down after 4 d of treatment. Therefore, it could be conjured that NO might be essentially required for the ABA-dependent proline accumulation under salt stress. But it was interesting that in the presence of SNP, exogenous ABA elevated the same level of proline compared with those of SNP treatment alone under salinity conditions. The proline

content was increased by 83.8 % in SNP treated wheat leaves under salt stress, but by ABA only 36.4%, which was much lower than those of SNP at 8 d. Whereas, when ABA was combined with exogenous NO treatment, the proline content was elevated by 80.2%, which was analogous with the results of NO treatment alone, indicating that NO induced analogous level of proline under salt stress no matter in the presence of ABA or not. Also, NO and ABA signaling displayed no synergism in proline induction (Fig.3B). Employment of hemoglobin combined with ABA furthermore illustrated the fact that NO might be downstream of the ABA-dependent proline accumulation under salinity conditions.

2.3 Effects of NO donor and ABA on P5CS and ProDH activities in wheat seedling leaves under salt stress

P5CS and ProDH appear to catalyze the rate-limiting steps in proline synthesis and degradation, respectively. Here, we principally tested the activities of P5CS and ProDH responding to exogenous NO donor and ABA treatment, respectively, under salt stress to uncover the reason of the higher proline accumulation induced by NO donor than ABA. As shown in Fig.4, SNP and ABA both elevated P5CS activities in wheat seedling leaves through 4 d of treatment, and an obvious higher level of induced P5CS activities by SNP were observed than those of ABA, which might contribute to the higher proline accumulation induced by NO donor than ABA did in the course of 4 d (Fig.3B). But after 4 d period, both of the regulation of SNP and ABA on P5CS activities disappeared and showed no statistical changes compared with those of 150 mmol/L NaCl salt stress alone.

Using the active staining method for ProDH, the single active band appeared (Fig.5). Stines *et al.* (1999) also reported that ProDH only existed in mitochondrial and the MW was about 55 kD. Surprisingly, the ProDH activity in wheat seedling leaves exhibited the enhancement within 2 d of exposure to 150 mmol/L NaCl salt stress than control, then reduced gradually by up to 8 d. Meanwhile, employment of SNP or ABA also induced the ProDH activities within 4 d of exposure to NaCl salt stress compared with NaCl treatment alone. After 4 d, ProDH activities were then depressed by SNP and ABA, respectively, and both of which might be the major reason to the accumulated proline levels after 4 d treatment by SNP and ABA (Fig.3B). It is worth mentioning that the level of inhibition to ProDH by ABA was obviously lower than than of SNP, particularly at 8 d of treatment, which might be responsible for the rapid accumulation of proline induced by NO donor under salt stress than those by ABA (Fig.3B). Taken together, it could be summarized that the enzymes responsible for proline accumulation under salinity conditions induced by NO

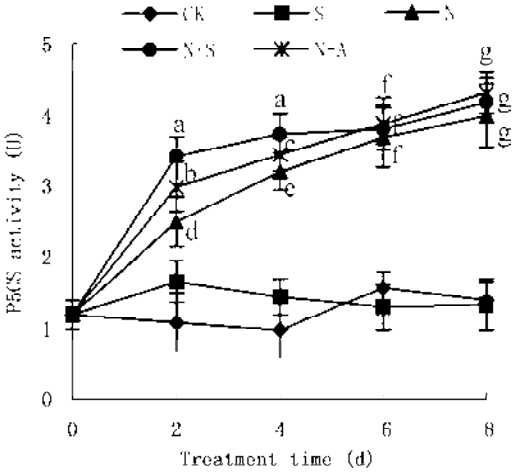

Fig.4. Effect of NO donor SNP and ABA on the regulation of P5CS activities in wheat seedling leaves under salt stress. Different treatment represents: Hoagland solution (control, CK), CK+0.1 mmol/L SNP (S), CK+150 mmol/L NaCl (N), CK+150 mmol/L NaCl+0.1 mmol/L SNP (N+S), CK+150 mmol/L NaCl+5 μmol/L ABA (N+A). Bars represent the mean ± *SE* of three independent experiments. One way ANOVA was used for comparisons between the means. Bars with different letters are significantly different at $P < 0.05$ or $P < 0.01$.

donor and ABA were different in a phase dependent manner. The weaker roles of ABA to P5CS and ProDH just explained the reason to the higher induced proline level by NO donor in wheat seedling leaves when subjected to 150 mmol/L NaCl salt stress (Fig.3B).

2.4 Role of Ca^{2+} in NO signaling pathway toward proline accumulation in wheat seedling leaves under salt stress

As shown in Fig 6, the NO induced proline accumulation was obviously decreased in wheat seedling leaves under 150 mmol/L NaCl salt stress when subjected to 10 μmol/L $LaCl_3$, a blocker of Ca^{2+} channel. The content of proline was decreased by 52.4% by $LaCl_3$ treatment in the presence of SNP, compared with SNP treatment alone under salt stress at 8 d. Contrary to this, when 50 mmol/L $CaSO_4$ was added to NaCl solutions, the proline content was basically elevated to the analogous level in comparison with SNP plus NaCl treatment except an obviously lower level at 8 d. Therefore, it could be concluded that Ca^{2+} might be tightly associated with the proline accumulation induced by NO in wheat seedling leaves when subjected to salt stress, and the signaling cascade of NO might be dependent on the cytosol $[Ca^{2+}]_c$.

3 Discussion

Salinity is one of the major factors limiting agricultural production around the world and promoting the salt

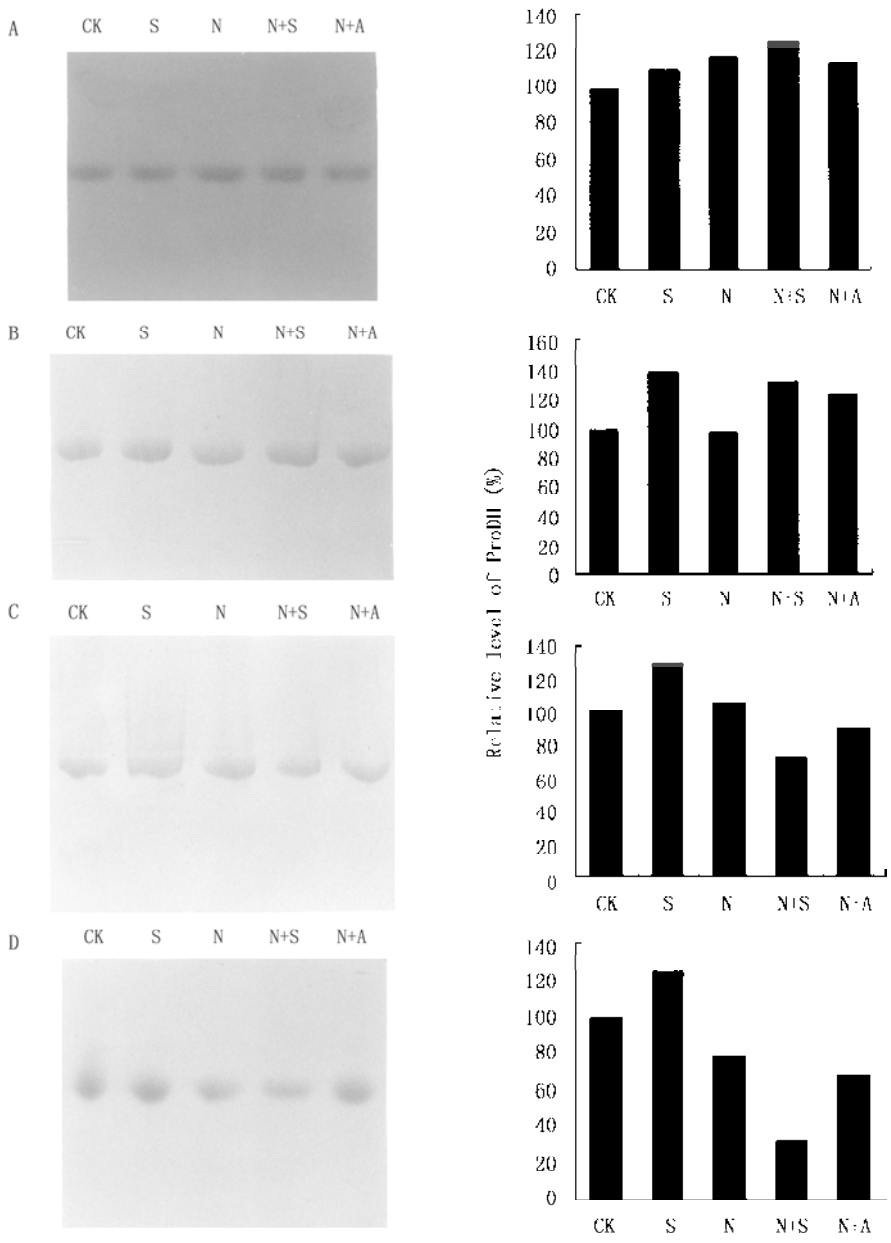

Fig.5. Changes of ProDH activities in wheat seedling leaves responding to NO donor and ABA under 150 mmol/L NaCl salt stress. **A**, **B**, **C** and **D** represent the results after 2, 4, 6 and 8 d treatment, respectively, and the corresponding chart at right side of the photographs were the relative ProDH activity level scanning with dual wavelength TLC scanner (CS-930) at 600 nm. Each lane was loaded equivalent amount of protein. The different treatments represent: Hoagland solution (control, CK), CK+0.1 mmol/L SNP (S), CK+150 mmol/L NaCl (N), CK+150 mmol/L NaCl+0.1 mmol/L SNP (N+S), CK+150 mmol/L NaCl+5 μmol/L ABA (N+A).

tolerance of crops has become an efficient pathway for agriculture. Meanwhile, overproduction of proline has been confirmed resulting in increased tolerance to osmotic or salt stress in transgenic plants (Kavi-Kishor *et al.*, 1995; Zhu, 2002), although there also existed the regards that proline was an alternative result from adaptive or detrimental processes responding to osmotic stress (Larher *et al.*, 2003). In present study, exogenous nitric oxide (NO) releaser SNP with different concentrations from 0.01 to 5 mmol/L induced proline accumulation in wheat seedling leaves under 150 mmol/L salt stress in a dose-dependent manner through 8 d of treatment (Fig.1A). Among of these, the effect of 0.1 mmol/L SNP was the most effective, while high concentration of SNP (5 mmol/L) had no obvious action (Fig.1A). Furthermore, the effect of SNP was specific for NO because the NO-scavenger c-PTIO and hemoglobin could reverse the effect of 0.1 mmol/L SNP on induced proline contents (Fig.1B). Meanwhile, the positive correlation between the enhanced percentage of RWC and proline induced by NO (Fig.2) ensured the speculation that the

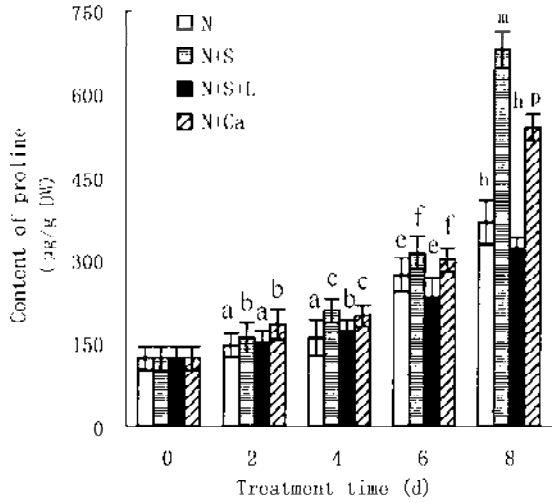

Fig.6. Effect of Ca^{2+} on the proline accumulation induced by NO in wheat seedling leaves under salt stress. Different treatment represents: CK+150 mmol/L NaCl (N), CK+150 mmol/L NaCl+0.1 mmol/L SNP (N+S), CK+150 mmol/L NaCl+0.1 mmol/L SNP+10 μmol/L $LaCl_3$ (N+S+L), CK+150 mmol/L NaCl+50 mmol/L $CaSO_4$ (N+Ca). Bars represent the mean±*SE* of three independent experiments. One way ANOVA was used for comparisons between the means. Bars with different letters are significantly different at $P < 0.05$ or $P < 0.01$.

induction of proline by NO might be an important mechanism for plants to tolerate severe saline conditions (Ruan *et al.*, 2002). Also in our experiments, the level of proline in wheat seedling roots were undetectable (data not shown) and the proline mainly existed in seedling leaves. It is valuable mentioning that we treated wheat seedling roots with NO releaser, but its eliciting effect on leaves might be due to the reason that NO was a permeable gas molecule, it could also transport over short and long distance with assistance of nitrite (Desikan *et al.*, 2002) and S-nitrosoglutathione (GSNO) (Reichenbach *et al.*, 2001). However, these related mechanisms have not been fully understood yet in plant tissues.

Recently, it has been suggested that there exists net cross talk between NO and ABA, cGMP, ion channels, Ca^{2+} and others in plants (García-Mata and Lamattina, 2002; Neill *et al.*, 2002; Correa-Aragunde *et al.*, 2004). The data in our experiments also showed that NO could activate the synthesis of endogenous ABA in wheat seedling leaves under 150 mmol/L NaCl salt stress (Fig.3A), which was in agreement with the results of Zhao *et al.* (2001) that the synthesis of ABA was inhibited by nitric oxide synthase (NOS) inhibitors in responding to drought stress.

Known to all, ABA has multiple roles in plants when subjected to salinity, including inducing the accumulation of proline. Also, proline accumulation appears to be mediated by both ABA-dependent and ABA-independent signaling pathway (Hare *et al.*, 1999). The result of Fig.3B illustrated that the level of proline was significantly elevated by exogenous supplied ABA in wheat seedling leaves under 150 mmol/L NaCl salt stress after 4 d of treatment ($P < 0.05$). Combination with hemoglobin treatment brought up with the fact that NO was a required factor downstream the ABA-induced proline accumulation, which was just in coincidence with the evidence that NO was a necessarily required component for the ABA induced stomatal closure (García-Mata and Lamattina, 2002; Neill *et al.*, 2002). Therefore, the maintenance of a high level of ABA by NO might be also an important aspect for the NO promoted salt tolerance following the reason that ABA has multiple roles in stress tolerance of plants, such as limiting water loss by inducing stomatal closure, promoting root morphogenesis and activating tolerant gene expression (Campalans *et al.*, 1999). Besides that, NO activated the synthesis of ABA and might also induce proline accumulation under salt stress via ABA transduction cascade, which supplied new evidence for good comprehension of cross talk between NO and ABA in plant kingdom. Additionally, the phytohormone ABA could enhance the synthesis of NO in reverse and induce closure of stomates, both NOS and nitrate reductase (NR) have been implicated in these processes (García-Mata and Lamattina, 2002; 2003; Neill *et al.*, 2002). Recently, Guo *et al.* (2003) identified a plant NOS gene playing a vital role in plant growth, fertility, stomatal movements and ABA signaling. Meanwhile, an inducible plant NOS gene was also been discovered by viral infection and encodes a variant of the P protein of glycine decarboxylase (GDC, Chandok *et al.*, 2003).

ABA and NO both activated the activities of P5CS and depressed the activities of ProDH in a phase dependent manner, mainly by enhancing the activities of P5CS within the first 4 d of treatment and inhibiting activities of ProDH 4 d later up to 8 d. And ABA showed a weaker effect on P5CS and ProDH in comparison with NO treatment (Figs.4, 5). These could be perfectly explained by above suggestion that NO might be downstream the ABA signaling pathway toward proline accumulation under salt stress (Fig. 3B). Uchida *et al.* (2002) also found that the expression of *P5CS* was increased in response to NO in rice seedlings leaves under salinity conditions, which could confer increased tolerance to salt stress. Additionally, our experiments of $LaCl_3$ and $CaSO_4$ (Fig.6) implicated the important role of Ca^{2+} in the pathway of NO in proline induction under salt stress. It was noted that plant *P5CS* and *ProDH*

might be co-ordinated by the same signaling cascade, involving both Ca^{2+} and cADPR (Hare *et al.*, 1999). cADPR also mediated the ABA signal transduction which elicited its effect via intracellular Ca^{2+} release, which was the core of NO signaling pathway in plants (Durner *et al.*, 1998). In a word, plants have developed delicate defense system to survive in nature.

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