Prime Scholars Library

Full Length Research Paper

Available online at
https://primescholarslibrary.org/

Advances in Agronomy, Plant Breeding and Horticulture

Vol. 6 (3), pp.37 - 43, June, 2018

The possible relationship between nitric oxide and the induction of nitrogen metabolism

M.O Oppong and P. N Akromah

Département of biological sciences, Faculté des sciences de Tunis, Université Tunis EL Manar 1060, Tunisia.

Abstract

The present work is focused on the possible relationship between nitric oxide and the induction of nitrogen metabolism in response to salt stress. The plants were subjected to 100 mM NaCl and sodium nitrite ($NaNO_2$) or sodium nitroprusside (SNP) as the NO donor. On one hand, plants showed lower Na^+ and Cl^- accumulation after application of SNP or $NaNO_2$ together with the NaCl treatment in leaves and roots of tomato. On the other hand, nitrate accumulation can be more important under SNP + NaCl and NO_2 + NaCl than NaCl treatment alone. Further results proved that NO significantly enhanced the activities of nitrate reductase (NR, EC 1.6.1.6) and glutamine synthetase (GS, EC 6.3.1.2), both of which separately contributed to the delay of ammonium accumulation in tomato plants under salt stress. Meanwhile, the glutamine synthetase (GS) activity was apparently enhanced by NO. Therefore, these results suggested that NO could strongly protect tomato plants from toxic damage caused by salt stress.

Keywords: *Solanum lycopersicom*, salt stress, nitric oxide, nitrogen metabolism.

INTRODUCTION

Accumulation of salts in irrigated soil is one of the primary factors limiting crop yield in the world (Rai and Rai, 2003). NaCl absorbed by the roots causes generally growth inhibition and imposes both ionic toxicity and osmotic stress to plants, leading to nutrition disorder (Serrano et al., 2002; Zhu, 2003). This results in accumu-lation of toxic levels of Na^+ and insufficient K^+ supply for enzymatic activities and osmotic adjustment (Zhu, 2003). Salt stress often leads to unfavorable functional changes and damage to plant tissues. It disturbs primordial meta-bolic pathways, including nitrogen metabolism (Debouba et al., 2007) and carbon assimilation (Delgado et al., 1993) leading to loss of energy and over-production of reactive oxygen species (ROS) (Mittler, 2002).

Improvement of plant tolerance to salt was shown to be related to compatible compounds accumulation (DiMartino et al., 2003), ability of cells to maintain hormonal balance (Kaya et al., 2009), nutrients homeostasis (Sanders, 2000) and sufficient salt ion compartmentalization (Binzel et al., 1988). These processes involve physiological, biochemical and molecular events that occur during salt stress (Debouba et al., 2006). Among these molecules, nitric oxide (NO) was regarded as an important signaling molecule in plants. It was claimed that NO is involved in plant development processes, such as germination (Liu et al., 2010), root organogenesis, stomatal closure (Garcia-Mata and Lamattina, 2002), leaf expansion and adaptative response to biotic and abiotic stresses like drought, salt, disease resistance and apoptosis (Besson-bard and Wendhenne, 2008; Tian and Lei, 2006; Zhao et al., 2004).

In the past, many plant biologists searched intensively for an NO-generating enzyme similar to the nitric oxide synthase (NOS) identified in mammalian systems (del Rio et al ., 2004). However, at present, the enzymatic source of NO in plant cells under normal or stress condi-tions is still a controversial issue (Crawford, 2006; Corpas et al., 2006; Neill et al., 2008; Moreau et al., 2008). NO production from nitrate reductase (NR) activity has been confirmed in plants (Yamazaki and Cohen, 2006). In plant

roots, NO can be generated by NR (Yamazaki and Sakihama, 2000) and nitrite:NO reductase (Ni:NOR) (Sthör et al., 2001).

Nevertheless, roles of NO on NR and nitrate nutrition in plants are not yet fully investigated. However, it is of necessity to assess the involvement of NO molecule within nitrate assimilation steps under control and stressed environment. For this purpose, tomato (*Solanum lycopersicon*) seedlings were cultivated in control and NaCl contaminated medium and effects of NO addition were evaluated in term of growth, nutrient acquisition and nitrogen assimilation.

MATERIALS AND METHODS

Plant material and growth condition

Tomato seeds (*S. lycopersicum* Mill. 'Chibli F1') were surface sterilized for 20 min in 20% (v/v) of calcium hypochlorite. Seeds were then germinated on moistened filter papers at 25°C in the dark. The seedlings thus obtained were transferred to pots (seven plants per 6 L) containing 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4, 0.5 mM $MgSO_4$, 32.9 mM Fe-K-EDTA and the micronutrients 30 mM H_3BO_4, 5 mM $MnSO_4$, 1 mM $CuSO_4$, 1 mM $ZnSO_4$ and 1 mM $(NH_4)_6Mo_7O_{24}$. The nutrient solutions were continuously aerated and renewed every 3 day to maintain pH (5.6 to 6) and nutrient composition. Plants were grown in a growth chamber: 26°C/70% relative humidity during the day and 20°C/90% relative humidity during the night; photoperiod is 16 h daily with a light irradiance of 150 µmol m^{-2} s^{-1} at the level of the plant canopy. Seedlings were grown in these conditions for 10 days, and then for 10 days in the medium containing 100 mM sodium chloride and 100 µM NO donors ($NaNO_2$ or SNP). Plants were harvested 6 h after the beginning of the light phase, and immediately separated into leaves and roots.

Ion analysis

Inorganic ions were extracted from dry materials with 0.5 N H_2SO_4 at room temperature for 48 h (Gouia et al., 1994). Sodium was analysed by flame emission using a spectrophotometer (Eppendorf, Netheler-Hinz, GmbH Hamburg, Germany). Chloride was quantified by a colorimetric method using a Digital Chloridometer (Haake-Buchler, Buchler Instruments Inc., NJ, USA). Nitrate was colorimetrically determined with an automatic analyser (Dual Tubingpump, Instrumenten B.V, Breda, The Netherlands) following diazotation of the nitrite obtained by reduction of NO_3^- on a cadmium column. Ammonium was extracted at 4°C with 0.3 mM H_2SO_4 and 0.5% (w/v) polyclar AT. The ammonium concentration was determined according to the reaction of Berthelot modified by Weatherburn (1967).

Enzyme assays

Determination of nitrate reductase

Nitrate reductase activity (NRA) was determined according to Robin (1979). Plant material was thoroughly washed with distilled water, and homogenized in a chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4), containing 7.5 mM cysteine, 1 mM EDTA, 1.5% (w/v) casein and 1% (w/v) polyvinyl-pyrrolidone (PVP). The homogenate was centrifuged at 30,000 g for 15 min at 4°C. The extract was incubated in a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.4), 10 mM EDTA, 0.15 mM NADH and 100 mM KNO_3 at 30°C for 30 min. The reaction was stopped by the addition of 100 µl of 1 M zinc acetate. Absorbance of the supernatant was determined at 540 nm after diazotation of nitrite ions with 5.8 mM sulfanilamide and 0.8 mM N-(1-naphthyl)-ethylene-diamine-dihydrochloride (NNEDD).

Determination of glutamine synthetase (GS)

Frozen samples were homogenized in a cold mortar and pestle with grinding medium containing 25 mM Tris–HCl buffer (pH 7.6), 1 mM $MgCl_2$, 1mM EDTA, 14 mM β-mercaptoethanol and 1% (w/v) polyvinyl-pyrrolidone. The homogenate was centrifuged at 25,000 g for 30 min at 4°C. Glutamine synthetase (GS) activity was determined using hydroxylamine as substrate, and the formation of γ glutamyl hydroxamate (γ-GHM) was determined with acidified ferric chloride (Wallsgrove et al., 1979). The γ-GHM was quantified using commercial glutamine as a standard after reading the absorbance of the incubation medium at 540 nm.

Enzyme assays

The data presented in this work are the average of at least five replicates per treatment; means ± S.E. are given in the figures. Each experiment was carried out in duplicate.

RESULTS

Sodium and chloride concentrations

Addition of 100 mM NaCl to nutrient solution induced an increase in Na^+ and Cl^- level in leaves and roots (Figure 1). However, addition of SNP or $NaNO_2$, to salt stress induced a decrease in Na^+ and Cl^- concentrations. Especially in leaves, this decrease is about 49 and 48% after addition of SNP and $NaNO_2$, respectively. A little decrease of these elements was also observed in roots.

Potassium and calcium concentrations

Figure 2 showed that the addition of 100 mM NaCl to nutrient solution reduced K^+ concentrations by 79% in leaves and 58% in roots (Figure 2), though Ca^{++} decreased after NaCl treatment for 10 days. On the other hand, addition of SNP to nutrient solution containing 100 mM NaCl increased K^+ concentrations in leaves from 0.31 to 1.33 mequiv.g^{-1} dry matter, and in roots from 0.75 to 1.99 mequiv.g^{-1} dry matter. When $NaNO_2$ was added to saline nutrient solution, K^+ increased significantly compared with plants treated only with NaCl. Other elements concentrations decreased with addition of 100 mM NaCl as Ca^{2+}, but the addition of NPS and $NaNO_2$ to saline nutrient solution either alone, increased root Ca^{2+} concentrations by 36.4 and 35.9%, respectively; also in leaves, the concentrations of Ca^{2+} is increased by 41.9 and 34.8%,respectively relative to the NaCl treatment.

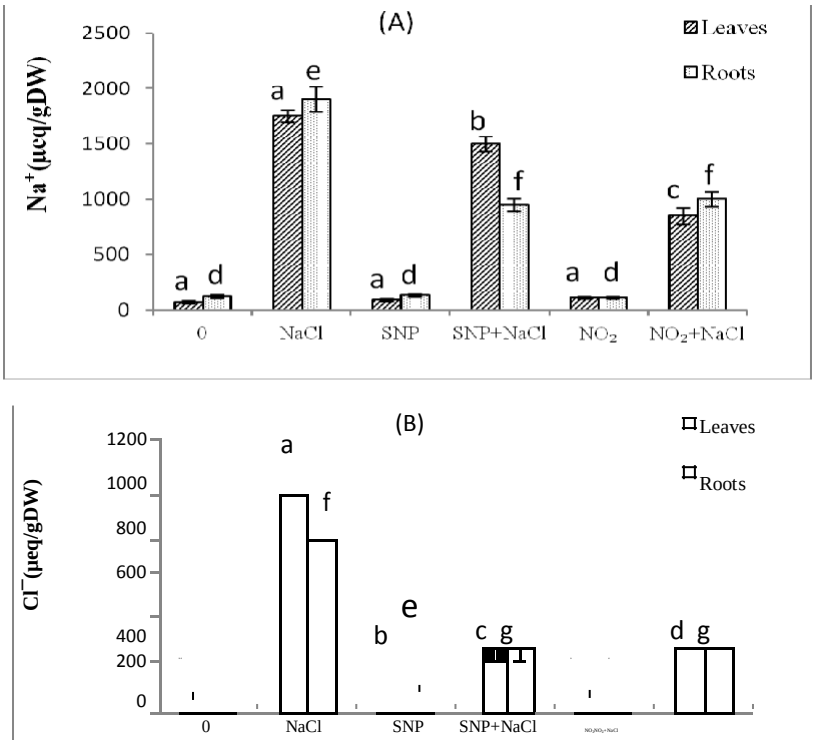

Figure 1. Effects of NaCl (100 mM) and NO (100 µM NPS or 100 µM $NaNO_2$) treatments for 10 days on contents of Na^+(A), Cl^-(B), in leaves and roots. Data are means of live replicates ± S.E.

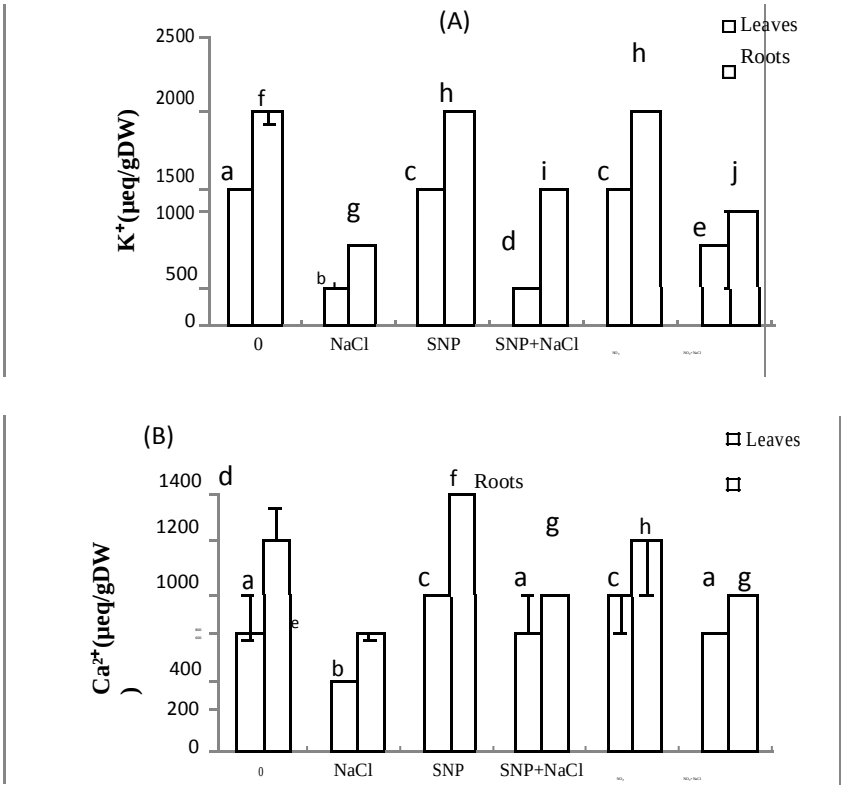

Figure 2. Effects of NaCl (100 mM) and NO (100 µM NPS or 100 µM $NaNO_2$) treatments for 10 days on contents of K^+ (A), Ca^{++} (B), in leaves and roots. Data are means of live replicates ± SE.

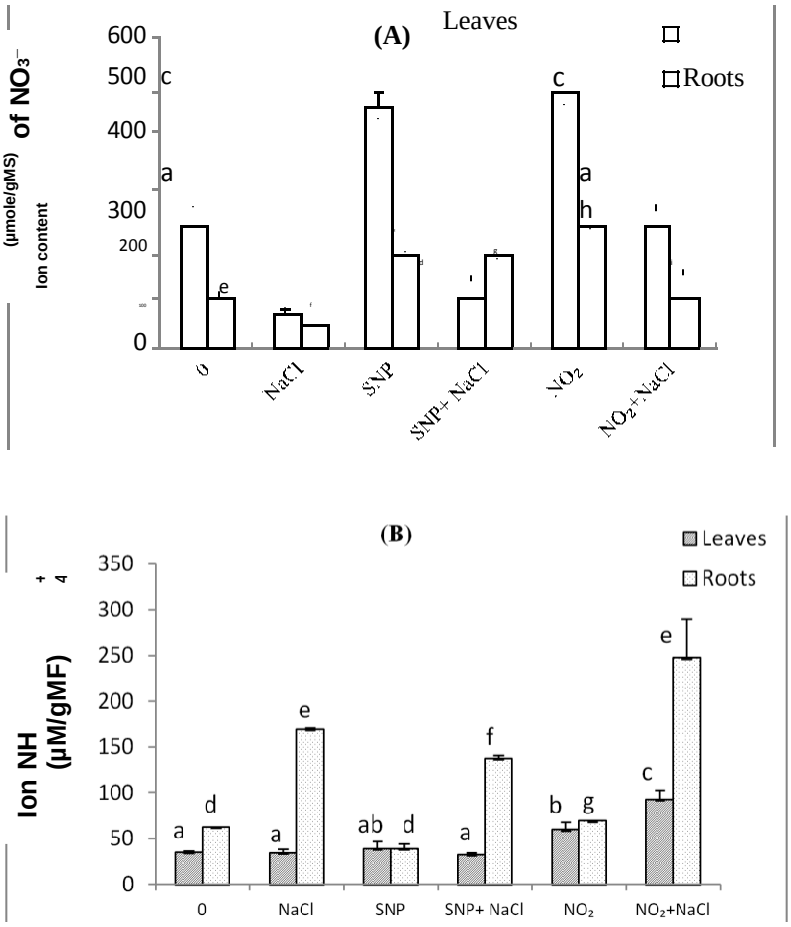

Figure 3. Changes during treatment of NO_3^- (A) and NH_4^+ (B) concentrations in leaves and roots of tomato. Plants were grown with (NaCl) or without (control) 100 mM NaCl and NO donor (100 µM NPS or 100 µM $NaNO_2$) for 10 days. Data are means of live replicates ± SE.

Nitrate and ammonium concentrations

The salinity treatments showed a decrease in nitrate accumulation in leaves and roots compared to the control (Figure 3). Adding SNP or $NaNO_2$ in the nutrient medium with salt induced a remarkable increase of nitrate which is more important in the case of roots, compared to salt treatment. In NPS and $NaNO_2$ treated plants, the nitrate level is higher than that of control plant. For plants treated with 100 µM $NaNO_2$, and 100 mM NaCl, a significant increase in NH_4^+ concentrations was recorded compared with plant treated only by 100 mM NaCl (Figure 3).

Nitrate reductase activity

Considering plants, NR is one of the NO former enzymes, it was possible that this important gaseous molecule was involved in the regulation of the NR activity. To characterize the effect of NO on tomato, either SNP or $NaNO_2$ were selected as NO donors. As it is shown, nitrate reductase activity (NRA) was considerably greater in leaves than in roots. Addition of 100 mM NaCl to nutrient solution induced a decrease in NRA by about 27% in leaves, and 15% in roots compared to the control (Figure 4). Due to the presence of NPS and $NaNO_2$, NR activity was considerably increased in leaves. However, NR activity was reduced in roots. When NO was added to saline nutrient solution, NR activity in leaves showed the same trends of that observed in the case of salt alone. NR activity of roots tomato treated with SNP or $NaNO_2$ in combination with NaCl was decreased significantly by about 82 and 86%, respectively, relative to the NaCl treatment.

Glutamine synthetase activity

The activity of GS in leaves and roots was assayed after increased in leaves and roots by 38.8 and 24.8%, respectively (Figure 5). After addition of $NaNO_2$, a similar response was observed in roots, but no significant change in GS activity was observed in leaves relative to the NaCl treatment.

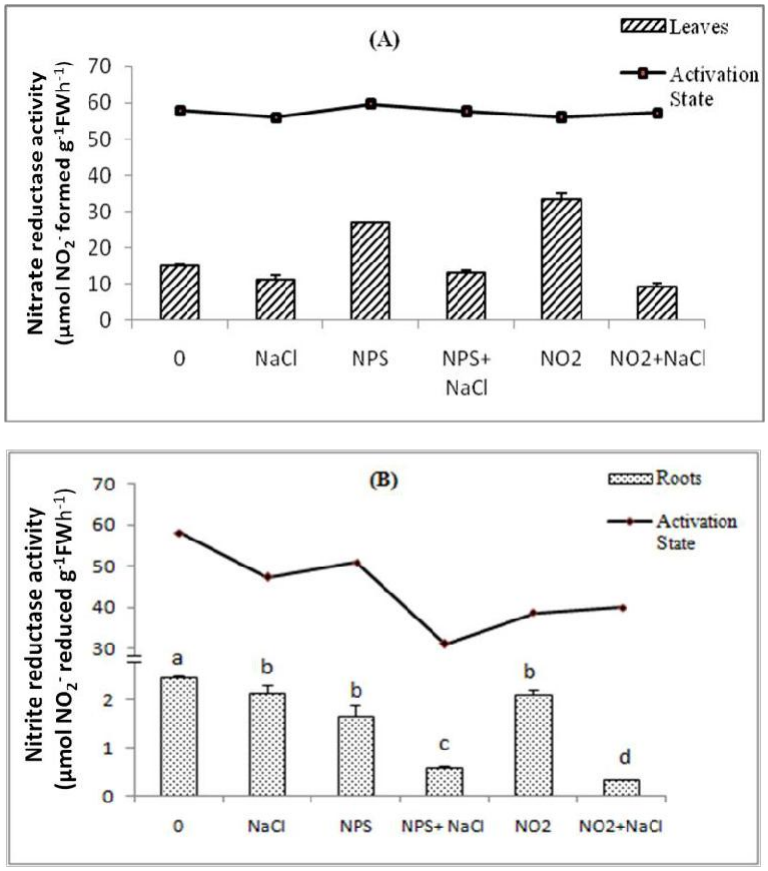

Figure 4. Changes during treatment of the maximal (EDTA) and activation state of nitrate reductase activity in the (A) leaves and (B) roots of tomato. Plants were grown with (NaCl) or without (control) 100 mM NaCl and NO donor (100 µM NPS or 100 µM $NaNO_2$) for 10 days. Data are means of live replicates ± SE.

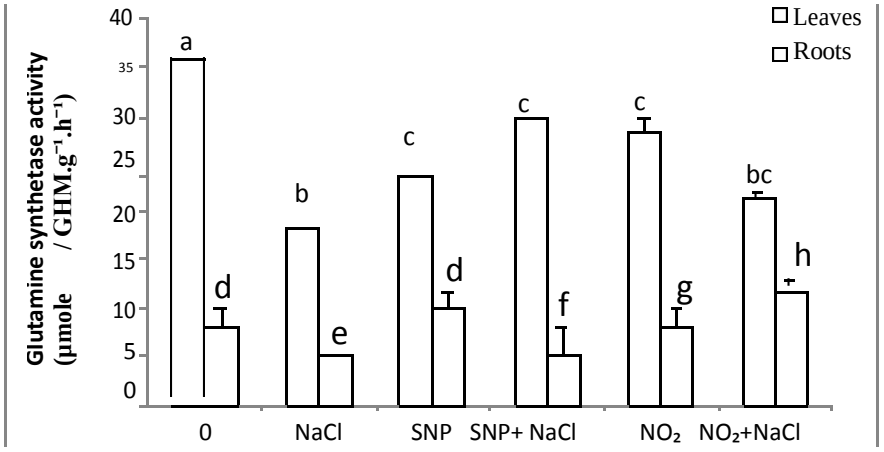

Figure 5. Changes during treatment of the glutamine synthetase activity in the leaves and roots of tomato. Plants were grown with (NaCl) or without (control) 100 mM NaCl and NO donor (100 µM NPS or 100 µM $NaNO_2$) for 10 days.

DISCUSSION

The involvement of NO in the mechanism of response to salinity has begun to be studied, although the data available can sometimes be contradictory, depending of plant species and the severity of the salinity treatment. The NO function in salt tolerance was demonstrated in many plant species such as Arabidopsis (Weihua et al.,

2009) and wheat (Ruan et al., 2002). In the present work, evidence was provided for the involvement of NO in moderating the toxic effect of salt. Ion analysis showed that at 100 mM NaCl, excessive accumulation of Na^+ and Cl^- concentrations was observed in leaves. Conse-quently, salt effect on DW production appeared remarka-bly in leaves indicating that critical levels of salt ions were reached; according to Debouba et al. (2006), the higher leaf growth salt sensitivity relative to roots, may be related to the highest Cl^- concentration relative to the roots (Figure 1). But it could be clearly seen that the concentrations of Na^+ and Cl^- of the roots and lea-ves were decreased both in the SNP and $NaNO_2$ treatments (Figure 1). Similar observations made by Zhao et al. (2004), also marked a decrease in Ca^{++} and K^+ concentrations observed in tomato plants under salt stress, and this effect was almost completely reverted when plants were pretreated with a NO donor for 10 days. Zhang et al. (2006) reported that NO induce an increase of PM H^+-ATPase to create electrochemical gradient for the establishment of ionic homeostasis to confer salt resistance. A recent study also revealed that NO alleviates salt toxicity in maize (Zhang et al., 2006). NO has also been shown to elicitate an increase in cytosolic Ca^{++} concentration through activation of intra-cellular Ca^{++} release (Garcia-Mata et al., 2003). The elevated cytosolic Ca^{++} activity may act as a messenger to modulate K^+ influx channels and high affinity K^+ transporters. Other studies, showed that both NO and NaCl treatment stimulated vacuolar H^+-ATPase and H^+-PPase activity resulting in increased H^+ translocation and Na^+/H^+ exchange (Zhu 2003; Zhang et al., 2006).

The nitrate concentrations in leaves and roots always decreased by 100 mM NaCl (Figure 3A), the decrease in NO_3^- contents can be attributed to an uptake compe-tition between Cl^- and NO_3^- within nitrate transporters (Frederico and Pedro, 1995), and/or an alteration of these transporters by toxic effects of Cl^-. However, our results showed that NO by itself increased nitrate concentration more than over the controls; also, addition of exogenous NO with NaCl significantly en-hanced nitrate concentration. The increase in NO_3^- could be due to an efflux from nitrates that previously exist in the mitochondria or chloroplasts, as occurred in nicotiana benthamiana where a rapid NO_3^- efflux was shown to be essential for NO production by NR and the subsequent defense responses induced by elicitin (Yamamoto et al., 2006).

Its well known that higher plants acquire the majority of their nitrogen by nitrate assimilation, and it has been suggested that NaCl-salinity is described to cause an inhibition on the growth of tomato plants by affecting N-metabolism (Debouba et al., 2006). Nitrate reductase (NR) catalyses the transfer of two electrons from NAD (P)H to nitrate to produce nitrite, which is further reduced to NH_4^+ by nitrite reductase. In this work, as for nitrate concentration, NR activity was inhibited by 100 mM NaCl in leaves than in roots. On the other hand, a positive correlation was found between NR activity and nitrate concentration in leaves and roots (Figures 3 and 4). In this sense, cytosolic nitrate seems to protect the NR enzymes against the action of proteases and/or inhibitors besides triggering the de novo synthesis of Nr protein by induction of NR gene expression (Campbell, 1999). Flores et al. (2000) showed that NR activity decrease by Cl^- in the tomato seedlings was due to a reduction in nitrate uptake, which decreased nitrate concentration in the leaves. Also, NR is highly regulated by light and dark transitions, and reversible protein phosphorylation.

When plants were treated with NO only, NR activity in leaves was positively modulated by NO released from SNP or $NaNO_2$; results are in accordance with those found by Du et al. (2008) in Chinese pakchoi cabbage, who reported that NR activity was significantly enhan-ced by the addition of the NO donors, and suggested that the effect of NO on NR activity might be due to an enhancement of electron transfer from haem to nitrate through activating the haem and molybdenum centers in the NR. But in roots these activity was slightly inhibited by NO donors. A similar effect for NO was des-cribed by Jin et al. (2009) in roots of tomato, suggesting that NO mediates the NR activity in plant roots depend-ing on the level of nitrate supply. When NO was applied together with salt, NR activity decreased in the leaves and roots. This results found that NO cannot alleviate the deleterious effect of salt on NR in tomato.

GS activity is a major enzyme participating in ammo-nium assimilation in higher plant. In the present study, treatment with 100 mM NaCl induced a decrease in GS activity in tomato leaves. Similar results were reported by Cramer et al. (1999). The decreased GS activity in leaves is a consequence of a reduced accumulation of the GS_2 isoenzyme, which in turn may partially be a consequence of post-translational regulatory mecha-nisms, which already have been decreased for several GS isoenzymes of other plant species. Addition of SNP or $NaNO_2$ to nutrient medium, slightly reduced GS activity in leaves (Figure 5). The reduction of glutamine synthetase activity by NO can be directly attributed to the increased GS nitration levels (Paula et al., 2011), suggesting that GS in post-translationally inactivated by NO-mediated nitration in response to lower nitrogen fixation rates. Given that the ammonium released by nitrogen fixation is assimilated in the cytosol by GS, it seems reasonable that the enzyme activity is modulated in response to the cell requirements to shut down ammonia assimilation if it is not being produced. In roots, GS activity was not influenced by exogenous NO application. NO-NaCl treatment did not exert any significant effect on the GS activity.

Conclusion

In tomato, treatment with NO donors (SNP or $NaNO_2$)

resulted in both the induction of nitrate uptake and the increase of nitrate reductase activity in the leaves, and similar effect regarding into mineral nutrition compared to plant control. It has been suggested that exogenous NO can induce physiological and cellular responses to salt stress. In the present work, we obtained experimental evidence indicating that exogenous NO is involved in prevention of Na^+ accumulation, and the increase of K^+ concentrations, also NO influence Ca^{++} absorption, and increase nitrate uptake. This reveals the protective role of NO in plant under salt stress. But, the nitrogen metabolism appeared not significantly ameliorated; indeed NR activity was decreased, when the GS activity showed similar values than in NaCl-treated plants.

REFERENCES

Besson-Bard A, Wendhenne D (2008). New in sights into nitric oxide signaling in plants. Ann. Rev. Plant Biol. 59:21-39.

Binzel ML, Hess FD, Bressan RA, Hasegawa PM (1988). Intracellular compartmentation of ions in salt adapted tobacco cells. Plant Physiol. 86:607-614.

Campbell WH (1999). Nitrate reductase structure, function and regulation: bridging the gap between Biochemistry and Physiology. Ann. Rev. Plant Physiol. Mol. Biol. 50:277-303.

Corpas FJ, Barrosso JB, Carreras A, Valderrama R, Palma JM, Leon AM, Sandalio LM, del Rio LA (2006). Constitutive arginine dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 224:246-254.

Cramer MD, Gao ZF, Lips SH (1999). The influence of dissolved inorganic carbon in the rhizosphere on carbon and nitrogen metabolism in salinity-treated tomato plants. New Phytol. 142:441-453.

Crawford NM (2006). Mechanisms for nitric oxide synthesis in plants. J. Exp. Bot. 57:471-478.

Debouba M, Maâroufi-Dghimi H, Suzuki A, Ghorbel MH, Gouia H (2007). Changes in Growth and Activity of Enzymes Involved in Nitrate Reduction and Ammonium Assimilation in Tomato Seedlings in Response to NaCl Stress. Ann. Bot. 99:1143-1151.

Debouba M, Gouia H, Suzuki A, Ghorbel MH (2006). NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato lycopersicon esculentum Seedlings. J. Plant Physiol. 163:1247-1258.

Delgado MJ, Garrido JM, Ligero F, Lluch C (1993). Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride stress. Physiologia Plantarum 89:824-829.

Del Rio LA, Corpas FJ, Barrosso JB (2004). Nitric oxide and nitric oxide synthase activity in plants. Phytochemistry 65:783-792.

Dimartino C, Sebastiano D, Pizzuto R, Loreto F, Fuggi A (2003). Free amino acid and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytologist. 158:455-463

Du S, Zhang Y, Lin XY, Wang Y, Tang CX (2008). Regulation of nitrate reductase by nitric oxide in Chinese cabbage pakchoi (*Brassica chinensis L.*). Plant Cell Environ. 31:195-204.

Flores P, Botella MA, Martinez V, Cerdá A (2000). Ionic and osmotic effects of nitrate reductase activity in tomato seedlings. J. Plant Physiol. 156(4):552-557.

Frederico GW, Pedro JA (1995). Characterization of the blue light-induced extracellular alkalinization associated with the monovalent anion uptake by *Monoraphidium braunii*: competition between NO_3^- and Cl^-. Physiol. Plant 94:545-552.

Garcia-Mata C, Gay R, Sokolovski S, Hilles A, Lamattina L, Blatt MR (2003). Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Porc. Natl. Acad. Sci. USA 10:11116-11121.

Garcia-Mata C, Lamattina L (2002). Nitric oxide and abscisic acid. Cross talk in guard cells. Plant Physiol. 128: 790-792.

Kaya C, Tuna AL, Yokas I (2009). The role of plant hormones in plants under salinity stress. Book salinity and water stress 44:45-50.

Gouia H, Ghorbel MH, Touraine B (1994). Effects of NaCl on flows of N and mineral ions and NO_3^- reduction rate within whole plants of salt sensitive bean and salt-tolerant cotton. Plant Physiol. 105:1409-1418.

Jin CW, Du ST, Zhang YS, Lin XY, Tang CX (2009). Differential regulatory role of nitric oxide in mediating nitrate reductase activity in roots of tomato (*Solanum lycocarpum*). Ann. Bot. 104:9-17.

Liu Y, Xu S, Ling T, Xu L, Shen W (2010). Heme oxygenase/carbon monoxide system participates in regulating wheat seed germination under osmotic stress involving the nitric oxide pathway J. Plant Physiol. 167: 1371-1379.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF (2008). AtNOS/ AtNOA1 is a functional *Arabidopsis thaliana* cGTPase and not a nitric-oxide synthase. J. Biol. Chem. 283:32957- 32967.

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Riberio D, Wilson I (2008). Nitric oxide, stomatal closure and abiotic stress. J. Exp. Bot. 59:165-178.

Paula MM, Liliana SS, Isa R, Ana RS, Helena C (2011). Glutamine Synthetase Is a Molecular Target of Nitric Oxide in Root Nodules of *Medicago truncatula* and Is Regulated by Tyrosine Nitration. Plant Physiol. 157:1505-1517.

Rai AK, Rai V (2003). Effect of NaCl on Growth, Nitrate Uptake and Reduction and Nitrogenase Activity of Azolla pinnata-Anabaene azollae. Plant Sci. 164:61-69.

Robin P (1979). Etude de quelques conditions d'extraction de la nitrate réductase des racines et des feuilles de plantules de maïs. Physiologie Végétale 17:45-54.

Ruan H, Shen W, YE M, Xu L (2002). Protective effects of nitric oxide on salt stress-induced oxidative damage to wheat (*Triticum aestivum* L.). leaves Chinese Science Bulltin, 47: 677-681.

Sanders D (2000). Plant biology: The salty tale of Arabidopsis. Curr. Biol. 10:486-488.

Serrano R, Rodriguez PL (2002). Plants, genes and ions. EMBO Rep. 3:116-119.

Sthör C, Frank S, Gisela M, Wolffram RU, Peter R (2001). A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nirate. Planta. 212: 835-841

Tian X, Lie Y (2006). Nitric oxide treatment alleviates drought stress in wheat seedlings. Biologia Plantarum 50(4):775-778.

Wallsgrove RM, Lea PJ, Miflin BJ (1979). Distribution of the enzymes of nitrogen assimilation within the pea leaf cell. Plant Physiol. 63:232-236.

Weatherburn MW (1967). Phenol-hypochlorite reaction for determination of ammonia Anal. Chem. 39:971-974

Weihua Q, Shouhua L, Fan LM (2009). Expression of a rice gene OsNOA1 re-establishes nitric oxide synthesis and stress-related gene expression for salt tolerance in Arabidopsis nitric oxide-associated 1 mutant Atnoa1. Environ. Exp. Bot., 65: 90-98.

Yamamoto-Katou A, Katou S, Yoshioka H, Doke N, Kawakita K (2006).
Nitrate reductase is responsible for elicitin-induced nitric oxide production in *Nicotiana benthamiana*. Plant Cell Physiol. 47:726-735.

Yamazaki H, Cohen MF (2006). NO signal at the crossroads: polyamine-induced nitric oxide synthesis in plants? Trends Plant Sci. 11:522-524.

Yamazaki H, Sakihama Y (2000). Simultaneous production of nitric oxide and per- oxonitrite by plant nitrite reductase: in vitro evidence for the NR-dependent formation of reactive nitrogen species. FEBS Lett. 468:89-92.

Zhang YY, Wang LL, Liu YL, Zhang Q, Wei QP, Zhang WH (2006). Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na^+/H^+ antiport in the tonoplast. Planta 224:545-555.

Zhao LQ, Zhang F, Guo JK, Yang YL, Li BB, Zhang LX (2004). Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. Plant Physiol. 134:849-857.

Zhu JK (2003). Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. 6:1-5.