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Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (*Solanum lycopersicum*) plants

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Abstract

Nitric oxide (NO) is involved in numerous physiological and stress responses in higher plants. Tomato is one of the most important vegetable crops in the world and previously it has been reported that salinity induced an oxidative stress affecting its redox and NO homeostasis. Using tomato plant exposed to 120 mM NaCl, it was studied whether the exogenous application of NO could ameliorate the negative effects provoked by salinity. Thus, nitric oxide provoked a significantly increase in the main antioxidative enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POD) activities, and also a raise of some enzymes involved in nitrogen metabolism including nitrate reductase (NR) and nitrite reductase (NiR) activities. Moreover, NO-treated plants showed a higher content in both proline and ascorbate but lower content of H_2O_2. These data indicate that the exogenous NO application is useful to mitigate the salinity-induced oxidative stress in tomato plants.

Keywords: Salt stress, tomato, nitric oxide, antioxidant system, nitrogen metabolism

1. Introduction

Salinity is an environmental stress which is constantly expanding around the world. In plants, salinity can induce damages in proteins, lipids and nucleic acids, and alterations in photosynthesis and respiration which affect plant growth and development. To overcome potential damages, higher plants have multiple protective mechanisms against salt stress including ion homeostasis, osmolyte biosynthesis, reactive oxygen species (ROS) scavenging, water transport and transducers of long-distance response coordination (Tuteja, 2007). In many cases, salinity stress contains an oxidative stress component due to the uncontrolled generation of ROS which provokes damage to macromolecules or even damage to the antioxidative system (Manaa *et al*., 2013). The antioxidative enzymatic system includes the enzymes superoxide dismutases (SOD), and catalase (CAT) and peroxidases which are involved in the detoxification of superoxide radical ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2), respectively, thereby preventing the formation of hydroxyl radicals ($^{\bullet}OH$) (Gill and Tuteja, 2010). Ascorbate peroxidase (APX), monodehydroasecorbate

reductase (MDAR) and glutathione reductase (GR), as well as glutathione (GSH) and ascorbate, are also important components of the ascorbate-glutathione cycle responsible for the removal of H_2O_2 in different cellular compartments (Foyer and Noctor, 2005). All these antioxidant components must work coordinately to provide a successful response against a specific stress.

Nitric oxide (NO) is one of the most studied bioactive molecules, due to its involvement in a wide spectrum of physiological processes from bacteria to human. In higher plants, NO has an important function in plant growth and development, in processes including seed germination, primary and lateral root growth, flowering, pollen tube growth regulation, fruit ripening, senescence, defence response and abiotic stresses, and NO is also a key signalling molecule in different intracellular processes (Nasibi and Kalantari, 2009; Corpas *et al*., 2011). The participation of NO in the mechanism of response to salinity stress has been also reported, although the information available can sometimes be contradictory, depending of the plant species and the severity of the salinity treatment (Zhao *et al.*, 2004; Begara-Morales *et al.*, 2014). Previously, we have demonstrated that salinity drastically affects redox and NO homeostasis in tomato roots (Manai *et al.*, 2014). Based in these data, the goal of the present work was to study whether the exogenous NO application throughout tomato roots could modulate the antioxidant defense in growing tomato plants under NaCl stress and evaluate also if this response could alleviate NaCl toxicity.

2. Materials and Methods

2.1. Plant material and growth condition

Tomato (*Solanum lycopersicum*, Mill. 'Chibli F1') seeds were surface sterilized with 20% (v/v) calcium hypochlorite solution for 20 min, and then were washed with distilled water, and germinated on moist filter papers at 25 ºC in the dark. Healthy and vigorous seedlings were selected and grown in aerated optimum-nutrient solutions 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 µM H_3BO_4, 5 µM $MnSO_4$, 1µM $CuSO_4$, 1 µM $ZnSO_4$ and 1 µM $(NH_4)_6Mo_7O_{24}$ for 8 days. Then, tomato plants were subjected for additional 8 d to stress with 120 mM NaCl either with or without 100 µM or 300 µM sodium nitroprusside (SNP) as NO donor. The growth chamber conditions were: 26 ºC/20 ºC (day/night), 70%-90% relative humidity (day/night), a 16 h photoperiod, and a light intensity of 150 µmol m^{-2} s^{-1}. Samples (leaves and roots) were harvested 6 h after the beginning of the light phase, immediately frozen in liquid nitrogen and stored at -80 ºC until further analysis.

2.2. Crude extracts

Leaves and roots from control and NaCl-treated tomato plants were ground in liquid nitrogen using a mortar and pestle. The resulting powder was added to 1/3 (w/v) extraction medium of 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 5% (v/v) polyvinylpoly-pyrrolidone (PVPP). For the ascorbate peroxidase (APX) assay, the extraction medium was also added with 5 mM ascorbate. The crude extracts were centrifuged at 14,000 g for 30 min at 4 ºC, and the supernatants were used for assays. For nitrate and nitrite reductase activities the extraction buffer was quite different. Thus, the resulting powder was added to 1/8 (w/v) extraction medium of 100 mM potassium phosphate buffer, pH 7.4 containing 1 mM EDTA, 7.5 mM cysteine and 2.5% (w/v) casein. The homogenate was centrifuged at 30,000 g for 15 min at 4 °C.

Protein content was determined spectrophotometrically at 595 nm as described by Bradford (1976) using bovine serum albumin (BSA) as standard.

2.3. Enzymatic activity assays

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm according to Beyer and Fridovich (1987). Catalase (EC 1.11.1.6) was

determined by measuring the disappearance of H_2O_2, as described by Aebi (1984). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by monitoring the initial ascorbate oxidation by H_2O_2 at 290 nm (Chen and Asada(1989). Glutathion reductase (GR, EC 1.6.4.2) activity was determined by following the NADPH oxidation at 340 nm as described by Rao *et al.* (1996). Peroxidase (POD, EC 1.11.1.7) activity was assayed using guaiacol as a substrate according to Nickel and Cunningham (1969) which is based in the increase in absorbance at 470 nm due to the guaiacol oxidation. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% (v/v) guaiacol, 1.0 mM H_2O_2 and 0.1 ml plant sample. The activity was expressed as U mg^{-1} protein.

Nitrate reductase (NR, EC 1.7.99.4) activity was determined according to the method of Robin (1979). Briefly, samples were incubated in a reaction mixture of 100 mM potassium phosphate buffer (pH 7.4) containing 10 mM EDTA, 0.15 mM NADH and 0.1 M KNO_3 for 30 min at 30 ºC. The reaction was stopped by adding 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). Nitrite reductase (NiR, EC 1.7.2.1) activity was measured according to Losada and Paneque (1971) based in the nitrite reduction at 540 nm. NiR activity was expressed as µmol NO_2^- • h^{-1} • g^{-1}FW).

2.4. Determination of ascorbate (ASC) content

ASC content was determined according to Hodges *et al.* (1996). Samples (0.3 g) were homogenized with 5% m-phosphoric acid (2 ml). The homogenate was centrifuged at 12,000 g for 20 min. Then, the supernatant (100 µl) was mixed with 500 µl KH_2PO_4 buffer (150 mM, pH 7.4) containing 5 m M EDTA, and color was developed by adding 400 µl 10% ((w/v) trichloroacetic acid, 400 µl 44% (v/v) o-phosphoric acid, 400 µl o-dipyridyl in 70% (v/v) ethanol and 200 µl of 0.03 % (w/v) $FeCl_3$. The reaction mixtures were incubated at 40 °C for 1 h and the absorbance was measured at 525 nm.

2.5. Determination of proline content

Free proline content was determined according to Bates *et al*. (1973). Samples (0.5 g) were homogenized with 3% sulphosalycylic acid. Homogenates were filtered through filter paper. After addition of 2 ml of acidic ninhydrin reagent (2.5 g ninhydrin/100 ml of solution containing glacial acetic acid, distilled water and orthophosphoric acid 85 % at a ratio of 6:3:1) and 1 ml glacial acetic acid, resulting mixture was incubated at 100 °C for 1 h in water bath. Reaction was then stopped using ice bath. The mixture was extracted with toluene, and the absorbance was measured at 520 nm.

2.6. Determination of H_2O_2 content, and lipid peroxidation

H_2O_2 content was determined according to Wolff (1994). The assay is based on ferrous ion oxidation in the presence of the ferric ion indicator xylenol orange. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content according to the method described by Cavalcanti *et al.* (2004). Samples (0.5 g) were homogenized with a mortar and pestle in 4 ml of 1% (w/v) trichloroacetic acid (TCA) at 4 °C. Homogenates were centrifuged at 12,000 g for 20 min. Supernatant (1 ml) was added to a reaction mixture (3 ml) containing 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 30 min and the reaction was stopped by quickly placing in an ice bath. The absorbance of the fraction was measured at 440, 532 and 600 nm.

2.7. Native polyacrylamide gel electrophoresis and isozyme staining

Native polyacrylamide gel electrophoresis (PAGE) was performed using a BioRad mini-gel system at 4 °C, 120 V for 90 min. For SOD, the tomato samples were subjected to native PAGE with 10% resolving gel and 4% stacking gels. SOD activity was assayed using the method of Beauchamp and Fridovich (1971).

2.8. Statistical analysis

Pair-wise analyses of variance (ANOVA) were used to detect differences among treatments, p-values < 0.05 were considered statiscally significant.

3. Results

3.1. Growth parameters and photosynthetic pigment contents

Table 1 shows the analysis of growth parameters of tomato plants. Salinity (120 mM NaCl) provoked a significant reduction in both leaf dry mass (53%) and root length (47%) but root dry mass was not affected. When SNP (NO donor) was added to NaCl-treated plants, significant amelioration was observed. Thus, 100µM and 300 µM SNP reduced the loss of leaf dry mass and the diminished of the root length. Again, root dry mass seemed to be not affected.

Table 2 shows the chlorophyll content of tomato plants exposed to NaCl (120 mM) and the effect of SNP treatment. Salinity caused clear leaf chlorosis symptoms because chlorophyll *a* and *b* content was both reduced 52%. However, when SNP was added the content of chlorophylls was less affected, especially with 300 µM SNP (22%).

3.2. Lipid peroxidation and H_2O_2 content

Figure 1 shows the effect of exogenous SNP on lipid oxidation and H_2O_2 content in 120 mM NaCl-treated tomato plants. Figure 1 (panels A and B) shows that salinity provoked an increase of 35% and 37% of lipid peroxidation in both leaves and roots, respectively. Upon applying 300 µM SNP to NaCl- stressed plants, lipid peroxidation was reduced significantly in leaves compared with NaCl stress only which suggests that this concentration of NO donor reduced the membrane oxidative damage provoked by NaCl. However, this effect was not observed with any concentration of SNP in roots. Figure 1 (panels C and D) illustrates that H_2O_2 content was reduced in both organs of tomato plants treated with 120 mM NaCl. On the other hand, any of the SNP treatments seem to revert this effect on H_2O_2 content in any of the analyzed organs.

Table 1. Effect of exogenous SNP (NO donor) on growth parameters in 120 mM NaCl-treated tomato plants. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

Treatments	Leaf dry mass (mg)	Root dry mass (mg)	Root length (cm)
0 mM NaCl	110 ± 15	26 ± 3.2	83.5 ± 8.3
120 mM NaCl	51 ± 4.7 [a]	22 ± 3.6	44 ± 4.8 [a]
120 mM NaCl + 100µM SNP	76 ± 7 [b]	25 ± 3.9	55 ± 3.9 [b]
120 mM NaCl + 300µM SNP	91 ± 11	25 2.4	63 ± 6

Table 2. Effect of exogenous SNP (NO donor) on chlorophyll content in leaves of 120 mM NaCl-treated tomato plants. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

Treatments	Chlorophyll *a* (mg · g^{-1} FW)	Chlorophyll *b* (mg · g^{-1} FW)	Total chlorophyll content (mg · g^{-1} FW)
0 mM NaCl	1.20 ± 0.06	0.35 ± 0.04	1.55 ± 0.05
120 mM NaCl	0.58 ± 0.01[a]	0.17 ± 0.02[a]	0.75 ± 0.06[a]
120 mM NaCl +100µM SNP	0.68 ± 0.01	0.19 ± 0.02	0.87 ± 0.05
120 mM NaCl +300µM SNP	0.94 ± 0.03[b]	0.27 ± 0.03[b]	1.21 ± 0.08[b]

3.3. Ascorbate and proline content

Table 3 shows the effect of exogenous SNP (NO donor) on ascorbate and proline content in leaves and roots of 120 mM NaCl-treated tomato plants. Salinity provoked that the ascorbate content diminished a 60% and 49% in leaves and roots, respectively. Upon applying either 100 µM or 300 µM SNP to NaCl stressed plants, the diminished ascorbate was less accentuated in both organs. On the other hand, salinity induces proline accumulation in leaves (22%) and roots (29%). Upon applying 300 µM SNP to NaCl stressed plants, the proline content increased 46% and 59% in leaves and roots, respectively.

3.4. Antioxidant enzyme activities

Figure 2 represents the effect of exogenous SNP (NO donor) on catalase and superoxide dismutase (SOD) activities in leaves and roots of 120 mM NaCl-treated tomato plants. Figure 2 (panels A and B) shows that catalase activity was not affected in leaves and roots of 120 mM NaCl-treated tomato plants. Upon applying SNP to NaCl stressed plants, it was observed a light activity increase in both organs with 100 µM SNP but no change was detected with 300 µM SNP. On the other hand, Fig. 2 (panels C and D) shows that SOD activity increased in both organs of 120 mM NaCl-treated tomato plants. Upon applying SNP to NaCl stressed plants, it was observed a significant increase of SOD activity which was higher with 300 µM SNP.

Considering that SOD enzyme has several SOD isoenzymes which can be distinguished by native PAGE, similar experiment was done to determine which SOD isoenzyme(s) is(are) most affected for SNP-treatment. Figure 3 (panels A and B) shows the presence of one Mn-SOD and two CuZn-SODs in both organs of control tomato plants. The CuZn-SODs were designated as I and II by mobility increasing in the gel. When plants were exposed to 120 mM NaCl treatment, it was observed that MnSOD was apparently not affected but both CuZnSOD (I and II) bands were clearly more intense in both organs . Upon applying SNP to NaCl-stressed plants, the MnSOD of both organs did not shown any significant changes. However, in roots the intensity of the band corresponding to CuZnSOD II increased significantly and appeared also a new CuZn-SOD band designated as III (Figure 3B).

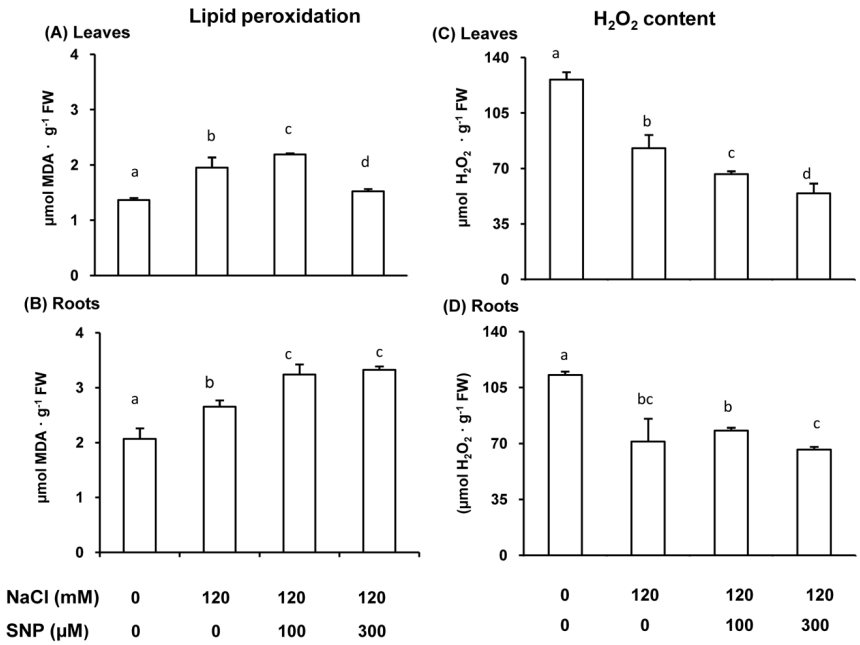

Figure 1. Oxidative stress parameters in leaves and roots of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 or 300 µM SNP). (A) Lipid peroxidation in leaves. (B) Lipid oxidation in roots. (C) Hydrogen peroxide content in leaves. (D) Hydrogen peroxide in roots. Data are the mean ± SEM of at least three different experiments. Different letters indicate that differences from control values were statistically significant ($p < 0.05$). FW, fresh weight.

Figure 4 shows the effect of exogenous SNP (NO donor) on APX and GR activities in leaves and roots of 120 mM NaCl-treated tomato plants. Figure 4A illustrates that APX activity was not affected in leaves of 120 mM NaCl-treated tomato plants; however in roots (Figure 4B) the APX activity increased (28 %). Upon applying SNP to NaCl-stressed plants, APX activity in leaves increased with 300 µM SNP but in roots the APX activity was enhanced by 45% and 53% with both concentration of NO donor. On the other hand , GR activity showed similar behavior in leaves (Figure 4C) and roots (Figure 4D) that the APX activity.

Figure 5A shows that peroxidase (POD) activity had a lightly increase but non-significant in leaves of of 120 mM NaCl-treated tomato plants, however in roots (Figure 5B) the POD activity increased (27 %) clearly. Upon applying SNP to NaCl-stressed plants, POD activity in leaves increased only with 100 µM SNP but in roots the POD activity was clearly enhanced being a 26% higher with 300 µM SNP.

Table 3. Effect of exogenous SNP (NO donor) on ascorbate and proline content in leaves and roots of 120 mM NaCl-treated tomato plants. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

Treatments	Ascorbate (mg · g^{-1} FW)		Proline (µg · g^{-1} DW)	
	Leaves	Roots	Leaves	Roots
0 mM NaCl	5.1 ± 0.3	3.7 ± 0.1	37.9 ± 2.0	20.2 ± 2.1
120 mM NaCl	2.1 ± 0.3^{a}	1.9 ± 0.2^{a}	49 ±5.8 ^{a}	28.1 ± 5.8^{a}
120 mM NaCl + 100µM SNP	3.0 ± 0.2^{b}	2.1 ± 0.2^{a}	52.1± 4.1^{a}	32.0 ± 4.1^{a}
120 mM NaCl + 300µM SNP	3.1 ± 0.5^{b}	2.3 ± 0.3^{b}	70.1 ± 3.2^{c}	48.8±3.2 ^{b}

3.5. Nitrate reductase (NR) and nitrite reductase (NiR) activities

Figure 6 shows the effect of exogenous SNP (NO donor) on NR and NiR activities in leaves and roots of 120 mM NaCl-treated tomato plants. Figure 6 (panels A and B) shows that NR activity was significantly inhibited in leaves (39%) and roots (19%) of 120 mM NaCl-treated tomato plants. Upon applying SNP to NaCl-stressed plants, it was observed a clear recovery of NR activity in leaves but being even higher in roots. Figure 6 (panels C and D) shows that NiR activity was not affected in leaves but was inhibited (30%) in roots of 120 mM NaCl-treated tomato plants. Upon applying SNP to NaCl-stressed plants, it was observed a clear increase of NiR activity in leaves with both concentration of NO donor but in roots it was not observed any recovery.

3. Discussion

Tomato fruit is one of the most consumed vegetables in the world either fresh or processed. However, tomato plants are also sensitive to different adverse environmental conditions including salinity which provoke a significant reduction in crop productivity (Tuteja, 2007). Many research efforts have been done in tomato plants to decipher the multiple mechanisms of response which could help to minimize the harmful salinity effects (Manai *et al.*, 2014; Manaa *et al.*, 2013). Previous data suggest that the exogenous application of NO has the capacity to improve plant tolerance to salinity, metal toxicity, temperature and drought stress in different plant species (Liu *et al.*, 2013; Wu *et al*., 2011; Esim and Atici, 2014). For example in the calluses of reed (*Phragmites communis*) under 200 mM NaCl, the addition of sodium nitroprusside, as NO donor, stimulated the expression of the plasma membrane H^{+}- ATPase, indicating that NO serves as a signal-inducing salt resistance by increasing the K^{+} to Na^{+} ratio (Zhao *et al.* 2004) and similar behavior was reported for maize (Zhang *et al*. 2006). Consequently, the goal of this study is to evaluate whether the exogenous NO application in tomato roots could also alleviate oxidative stress damages provoked for salinity stress in tomato plants.

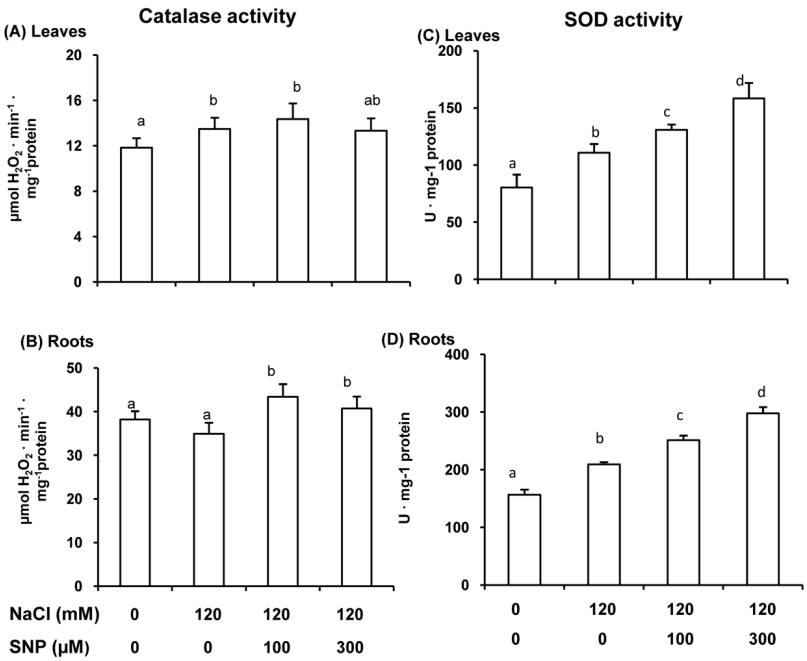

Figure 2. Catalase and superoxide dismutase (SOD) activities in leaves and roots of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 or 300 µM SNP). (A) Catalase activity in leaves. (B) Catalase activity in roots. (C) SOD activity in leaves. (D) SOD activity in roots. Data are the mean ± SEM of at least three different experiments. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

Under our experimental salinity conditions (120 mM NaCl), tomato plants were observed to be affected in relation to the analyzed growth parameters. Moreover, this salinity stress was also accompanied by an oxidative stress as observed by the increase in lipid peroxidation (a well recognized oxidative stress biomarker) and a general stimulation of the antioxidative system such as SOD, APX or POD activities. All these data are in good agreement with previous data reported on tomato plants under salinity stress (Manaa *et al.*, 2013; Manai *et al*., 2014). Moreover, similar behavior was observed in the case of both enzymes involved in nitrogen metabolism because both NR and NiR activities were negatively modulated by salt stress (Debouba *et al*., 2007).

When the NO donor (100 µM or 300 µM SNP) was added through the tomato root system, it was observed a clear recover in leaf dray mass, root length and chlorophyll content in leaves, suggesting the beneficial effects of this NO donor.

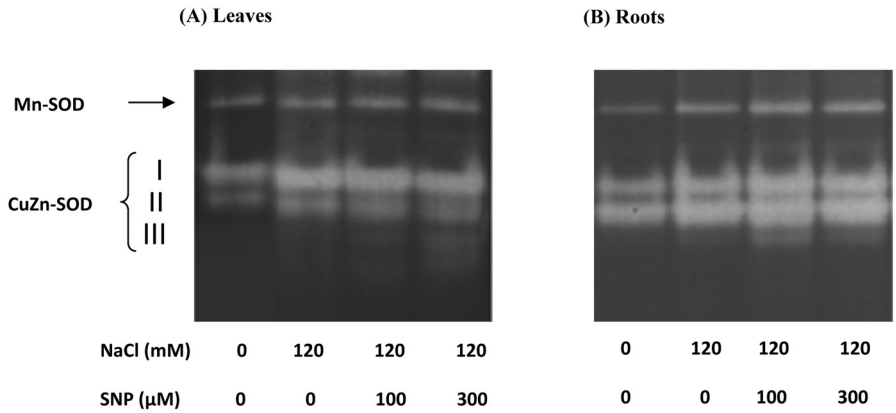

Figure 3. Superoxide dismutase (SOD) isoenzymes activities in native gels in leaves (A) and roots (B) of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 μM or 300 μM SNP).

These data are in also in good agreement with previous reports where NO seems to protect photosynthesis from damage induced by other stresses such as heat stress (Song *et al*., 2013). Similarly, recent evidence in plants reveals that exogenous NO treatments protect tomato cells from oxidation damage under stress by enhancing the antioxidant enzymes (Nasibi and Kalantari, 2009). The exogenous NO, furthermore, has been reported to alleviate the oxidative damage of salinity to seedling of rice (Wu *et al.*, 2011). NO can induce secondary metabolite accumulation and promote cell death (Bellin *et al*., 2013). In our experimental system, it was observed a general stimulation of the antioxidative systems. Thus, the application of SNP throughout the roots provokes a general increase in superoxide dismutase, ascorbate peroxidase, glutathione reductase and peroxidase activities in both roots and leaves. A remarkable result was the induction of a new CuZnSOD (III) in roots which suggests that NO donor exerts a regulation at protein and gene level of this antioxidant enzyme. Moreover, these data suggest also the existence of a signaling pathway initiated by NO which effects can be observed in leaves.

In plants NR is a key enzyme of nitrogen assimilation, catalyzing the reduction of nitrate to nitrite, which is further reduced to ammonium by the nitrite reductase (NiR) and then incorporated into amino acids and other nitrogen-derived compounds. In addition, *in vitro* evidence suggests that NR plays a pivotal role in NO synthesis in plants (Yamasaki and Sakihama, 2000). Thus, NR-mediated NO production was induced by various abiotic factors, such as osmotic stress (Nasibi and Kalantari, 2009), and salt stress (Wu *et al.,* 2011). However, it has been also described that NO can regulate NR activity (Du *et al.*, 2008). In our experimental system, it was observed that the exogenous NO application throughout the root system stimulated the NR activity in both roots and leaves which alleviates the negative effect of salinity stress. These data indicate a clear interrelationship between NO and nitrate reductase which allows the nitrogen assimilation during salinity stress.

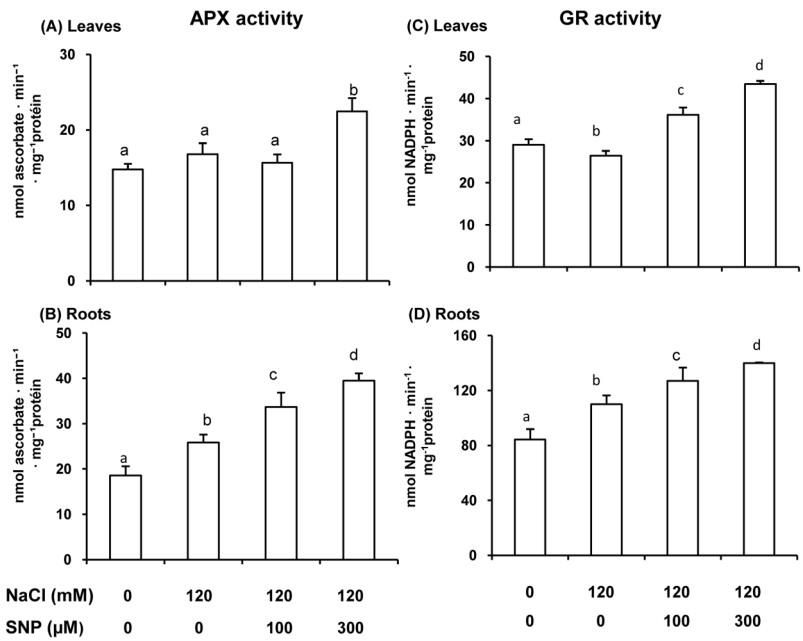

Figure 4. Ascorbate peroxidase (APX) and glutathione reductase (GR) activities in leaves and roots of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 or 300 µM SNP). (A) APX activity in leaves. (B) APX activity in roots. (C) GR activity in leaves. (D) GR activity in roots. Data are the mean ± SEM of at least three different experiments. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

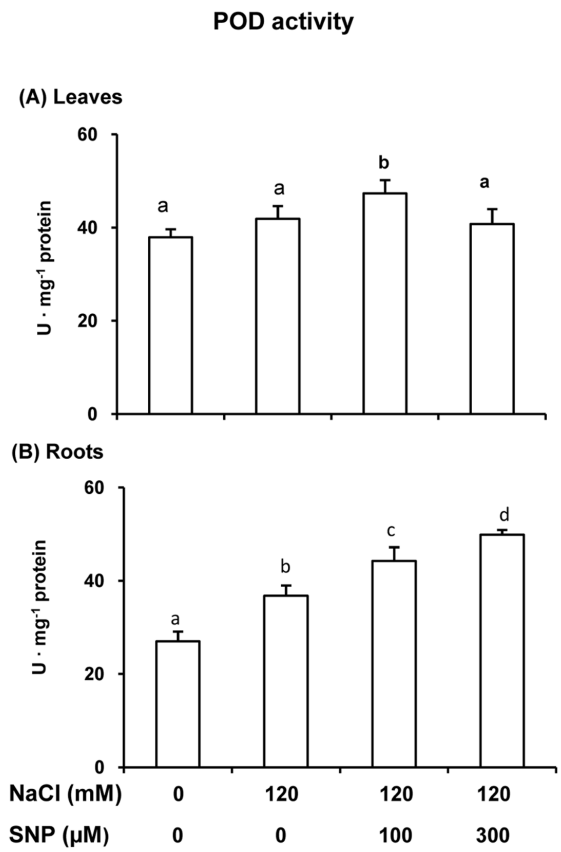

Figure 5. Peroxidase (POD) activity in leaves (A) and roots (B) of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 or 300 μM SNP). Data are the mean ± SEM of at least three different experiments. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

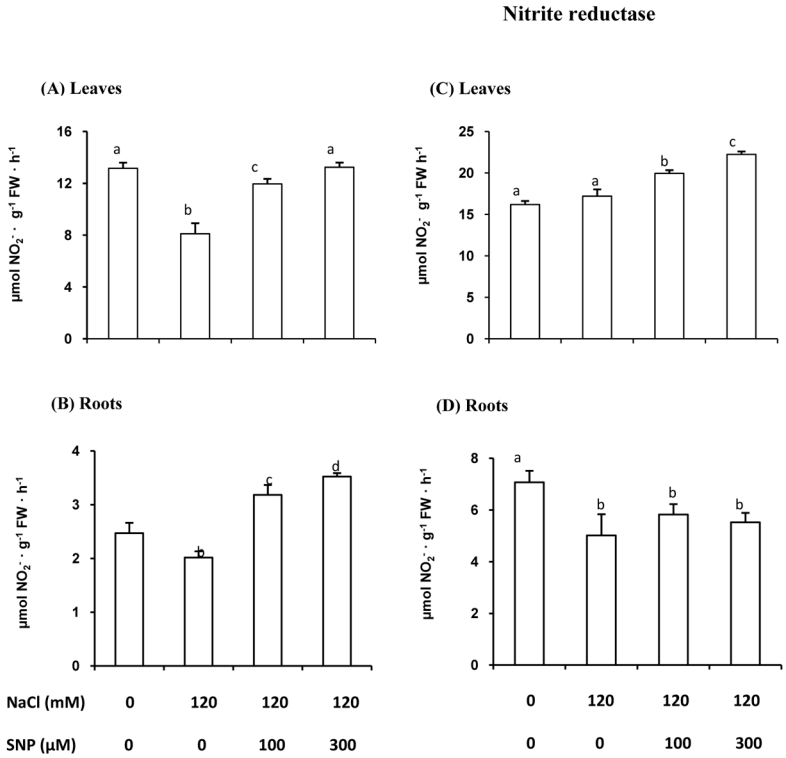

Figure 6. Nitrate reductase (NR) and nitrite reudctase (NiR) activities in leaves and roots of 120 mM NaCl-treated tomato plants supplemented with a NO donor (100 or 300 µM SNP). (A) NR activity in leaves. (B) NR activity in roots. (C) NiR activity in leaves. (D) NiR activity in roots. Data are the mean ± SEM of at least three different experiments. Different letters indicate that differences from control values were statistically significant ($p < 0.05$).

5. Conclusion

Taken together, the results reveal that adding SNP through the roots had significant beneficial effects against NaCl toxicity in tomato plants, which was reflected in the growth and chlorophyll content in NaCl exposed plants. Exogenous SNP decreased also NaCl-induced lipid oxidation levels in leaves, and induced the increase of activities of the antioxidant system including SOD, APX, GR and POD in roots and leaves plus the content of ascorbate and proline. In summary, the data suggest that the exogenous application of NO could contribute to ameliorate salinity-induced oxidative stress in tomato plants. Future research in this area under field conditions could contribute to develop a sustainable crop production in salinity contaminated areas.

Abbreviations

APX, ascorbate peroxidase; ASC, ascorbate; CAT, catalase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; MDA - malondialdehyde; NBT, nitroblue tetrazolium chloride; NO, nitric oxide; nitrite reductase (NiR); nitrate reductase (NR); POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; SNP, sodium nitroprusside.

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