Nitric oxide ameliorates salinity tolerance in Pyrodwarf pear (*Pyrus communis*) rootstocks by regulating polyamine content

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Abstract

Nitric oxide (NO), an endogenous signaling molecule, is involved in various physiological processes and stress responses in plants. In the present research, Pyrodwarf pear (*Pyrus communis*) rootstocks were grown by nutrient solution to investigate the effects of sodium nitroprusside (SNP) application as an NO donor at 0, 0.1, 0.5, and 1 mM levels on plant stress tolerance, content of main polyamines, physiological reactions, and activity of CAT and APX enzymes under NaCl stress condition at 0, 50, 100, and 150 mM concentrations. Exogenous NO significantly increased endogenous NO content of leaves, height of the plants and relative water content (RWC) of leaves while it decreased malondialdehyde (MDA) content. NaCl stress increased the content of putrescine (Put) and spermine (Spm), and exogenous NO resulted in a further increase the content of Spm in plant leaves under NaCl stress. Pyrodwarf pear rootstocks significantly showed higher content of proline and antioxidant activity under NaCl stress, and NO treatment further increased the content of proline and antioxidant activity in plants exposed to NaCl stress. The activity of antioxidant enzymes including catalase (CAT) and peroxidase (POD) increased in plants under NaCl stress, and exogenous NO further induced those antioxidant enzyme activities. Exogenous NO resulted in the enhancement of Spm content and plants with higher Spm content exhibited high levels of antioxidant activity and proline content under NaCl stress, indicating the beneficial effects of exogenous NO under salinity conditions. NO improved the tolerance of Pyrodwarf pear rootstocks under NaCl stress by regulating the content and the ratio of polyamines.

Keywords: NaCl; nitric oxide; pear; polyamines; rootstock

Yousefi, M., L. Naseri and F. Zaare-Nahandi. 2019. 'Nitric oxide ameliorates salinity tolerance in pyrodwarf pear (*Pyrus communis*) rootstocks by regulating polyamine content'. *Iranian Journal of Plant Physiology,*10 (1), 3023-3033.

Introduction

Salinity is a major restriction for the cultivation of horticultural crops. Pear trees are commonly sensitive to salinity stress (Maas, 1993). Pear rootstocks affect the nutritional status of the scion and the appropriate selection of rootstocks can decrease the damaging effects of salinity (Musacchi et al., 2006). NaCl stress at 100 mM level during 5 weeks did not have a significant effect on *Pyrus betulifolia* and its survival rate was 80% under 150 mM NaCl, but *Pyrus pyrifolia* was damaged under 25 mM NaCl irrigation (Okubo and Sakuratani, 2000). Irrigation with a salinity level of

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Received: August, 2019
Accepted: November 2019

about 5 dSm^{-1} caused only a slightly diminished growth of European pear (*Pyrus communis*) plants regardless of the genotype, indicating the relative tolerance of pear rootstocks (*P. communis*) under salinity stress for a short duration (Musacchi et al., 2006). Pear trees are commonly cultivated in low-salinity areas. However, salinity problems may increase under particular conditions, including cultivation in coastal areas and usage of irrigation water or fertigation (Musacchi et al., 2006). Salty waters can be used successfully for irrigation, in the area with fresh water deficiency (Rhoades, 1992). Plants have a variety of protective mechanisms to overcome the damage of proteins under salinity stress, including ionic balance, osmolytes biosynthesis, removal of reactive oxygen species (ROS), alteration in membrane structure and induction of antioxidant activity (Tuteja and Sopory, 2008).

NO is an important endogenous bioactive signaling molecule that has a key function in various processes of plant growth and development, including seed dormancy, seed germination, and lateral root growth, floral transition, flowering, pollen tube growth regulation, fruit ripening, photosynthesis, senescence, plant metabolism and cell death, aging, cellular protection against toxic ROS, defense reactions, and abiotic stresses as well as stress responses (Corpas et al., 2007; Lamattina et al., 2003; Manai et al., 2014). NO plays a pivotal role in stress tolerance exerted by oxidative stress (Ahmad et al., 2016). The application of sodium nitroprusside (SNP) as an NO donor, can enhance the plant growth under salinity stress by protecting it against oxidative damage, improving ionic balance, and inducing antioxidant compounds (Uchida et al., 2002; Zhang et al., 2006). Different concentrations of exogenous NO resulted in differential changes in polyamines and proline levels through their metabolic adjustment (Filippou et al., 2013). Protective role of NO is dependent on its ability to maintain cellular oxidation equilibrium and toxicity of ROS in plants under stress conditions (Hayat et al., 2010). Polyamines include putrescine (Put, a diamine), spermidine (Spd, a triamine), and spermine (Spm, a tetramine) which are involved in the regulation of many pivotal processes of the cells, including DNA replication, transcription, translation, cell division, enzymes' activity, cellular cation-anion balance, membrane stability, floral development, fruit ripening, secondary metabolism, aging and response to abiotic and biotic stresses (Bachrach, 2010). Polyamines are ubiquitous low molecular weight aliphatic amines that exist widely in plants and are involved in the regulation of plant growth and development. Due to their polycationic nature at physiological pH levels, polyamines are able to interact with proteins, nucleic acids, membrane phospholipids, and cell wall constituents (Fan et al., 2013). The value of polyamines' correlation with NO due to arginine is a common precursor in their biosynthesis (Gao et al., 2009). Polyamines induce the production of NO in *Arabidopsis thaliana* and this shows that NO could correlate with polyamines involved in stress response (Groppa and Benavides, 2008).

In this study, the effects of exogenous NO treatment on plant tolerance parameters, the content of main polyamines, interaction of NO with polyamines, and antioxidant parameters under NaCl stress were investigated in order to evaluate the tolerance of Pyrodwarf pear (*Pyrus communis*) rootstocks under NaCl stress.

Materials and Methods

Plant material and growth condition

The Pyrodwarf pear rootstocks (prepared from micro-propagated method), approximately 30 cm in height were transferred to the plastic pots with 26 cm diameter and a height of 27 cm containing a mixture of perlite and vermiculite medium (1:1) under the conditions of hydroponic greenhouse system with Hoagland nutrient solution. The experiment was conducted as a factorial based on a completely randomized design with three replicates for each treatment, and a total of 48 plants. SNP (n NO donor) treatments were applied after enough vegetative growth, increment of rootstock height about 20 centimeters, and extension of the root system of rootstocks in the pot, and then NaCl salinity was treated, 48 hours later. SNP treatments were applied at four levels of 0, 0.1, 0.5, and 1 mM along with nutrient solution through the root with the interval of 2 weeks in three steps and NaCl salinity treatments were applied at 0, 50, 100, and 150 mM concentrations along with nutrient solution

through the root system until sampling for 7 weeks. To measure the height of pear rootstocks, the height difference of pear rootstocks was measured before application of SNP treatment and NaCl stress, as well as at the end of the experiment, and expressed as the increment of rootstock height of the pear rootstocks.

Determination of endogenous NO content

Nitric oxide content was estimated by measuring NO_2^- anion in extracts by the Griess reaction (Green et al., 1982). Briefly, 100 µL aliquots of extracts were incubated with an equal volume of Griess reagent (0.1% N-(1-napththyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 5% phosphoric acid) (Sigma, St Louis, MO, USA). They were incubated for 15 min at room temperature. The developed color was read at 540 nm immediately using Griess reagent and extraction buffer as blank. Nitrite concentrations were determined on the basis of a standard curve prepared with sodium nitrite.

Measurement of lipid peroxidation and RWC

Lipid peroxidation in shoots after the two stresses was determined as the amount of MDA as measured by the thiobarbituric acid (TBA) reaction, according to the method documented by (Liu et al., 2006). Leaf relative water content (RWC) was assayed using leaf discs (5 mm in diameter). The fresh weight (FW) of leaf discs was weighed in each treatment. The leaf discs were held in distilled water until turgor weight (TW) for 24 hours. Dry weight (DW) was obtained after drying the leaf discs at 75 °C for 48 hours (Repellin et al., 1997). RWC was calculated according to the following formula:

$$RWC\ (\%) = (FW\text{-}DW)/(TW\text{-}DW) \times 100.$$

Polyamines analysis

Leaf samples were rinsed several times with distilled water. A total of 5 g FW of leaf tissue was homogenized in 5% (v/v) HClO4 to give a final concentration of 0.1 g mL^{-1}, using a mortar and pestle. The homogenate was centrifuged for 20 min at 18000 g, and the supernatant was used for polyamine analysis. A total of 0.5 ml of the extract was added to 2 ml of 4 N NaOH and 5 µl benzoyl chloride. The mixture was vortexed for 15 s and incubated in a water bath for 30 min at 35 °C. The benzoylation reaction was terminated by adding 4 ml concentrated NaCl, and the benzoylated polyamines were extracted with 4 ml chilled diethyl. The ether fraction was collected and centrifuged at 1500 g at 4 °C for 10 min. An aliquot of 2 ml of the ether fraction was brought to dryness in a rotary evaporator at room temperature. The derivate polyamines were re-suspended in 200 µl chilled acetonitrile. A 10 µl aliquot was injected into a Knauer HPLC system equipped with a UV detector set at 254 nm wavelength and a C- 18 reverse phase column (Eurospher 100-5 250 × 4.6 mm, Knauer, Berlin, Germany). Acetonitrile/ water (1:1 v/v) was used as the mobile phase in an isocratic mode (Flores and Galston, 1982).

Determination of proline content

The proline concentration was determined according to the modified method detailed by Bates et al. (1973). Proline concentration was determined using a standard curve and calculated on a fresh weight basis (mmol proline/g FW).

Measurement of DPPH radical scavenging activity and CAT and POD enzymes activities

The ability of OH69 pear rootstock leaves extract to scavenge DPPH free radicals was determined with the method of Brand-Williams et al. (1995). Leaves were homogenized in 0.1 M potassium phosphate buffer (pH 7) containing insoluble PVP and EDTA. POD activity was assayed according to (Chance and Maehly, 1955). One unit of activity was defined as the amount of enzyme required to increase 0.01 absorbance unit in the optical density at 470 nm in 1 min. CAT activity was carried out according to (Aebi, 1984). The change of absorbance at 240 nm was measured at 25° C. One unit of CAT activity was defined as the amount of the enzyme decomposing 1 µmol H_2O_2 min^{-1}.

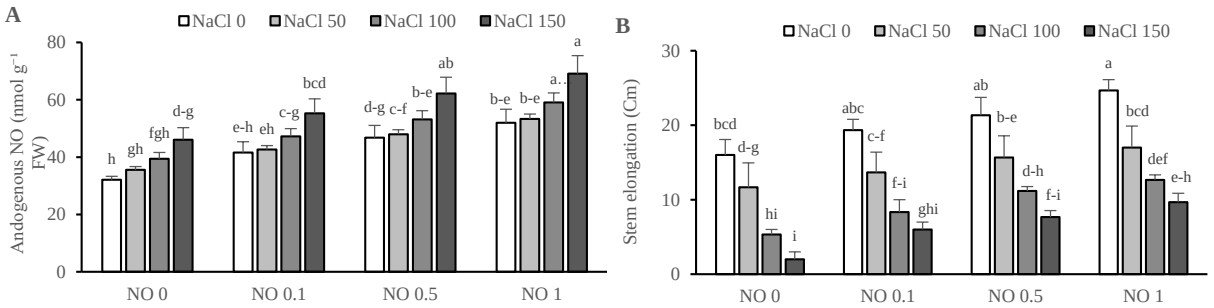

Fig. I. Effect of exogenous NO treatment at 0, 0.1, 0.5, and 1 mM on the endogenous NO (A) and stem elongation of height rootstock (B) in leaves of pyrodwarf pear rootstocks under NaCl stress at 0, 50, 100, and 150 mM concentrations; different letters indicate significant differences according to Duncan's test (P ≤ 0.05).

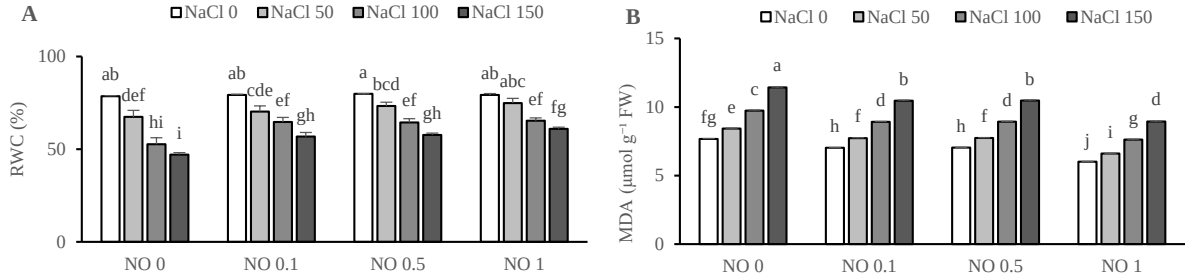

Fig. II. Effect of exogenous NO treatment at 0, 0.1, 0.5, and 1 mM on the relative water content (RWC) (A) and MDA content (B) in leaves of pyrodwarf pear rootstocks under NaCl stress at 0, 50, 100, and 150 mM concentrations; different letters indicate significant differences according to Duncan's test (P ≤ 0.05).

Statistical Analysis

After data collection, the analysis of variance was performed. To test the significant differences among treatments, the general linear model (GLM) was applied. The significance of the differences were tested using Duncan's New Multiple Range Test (DMRT) at P≤0.05 and considered to be statistically significant at 95%. All statistical analyses were done using SPSS version 22 software for Windows (IBM SPSS Statistics release 22.0.0, 2013, SPSS Inc., Chicago, IL, USA).

Results

Endogenous NO content

Results showed that the content of endogenous NO increased by increasing NaCl stress in leaves of Pyrodwarf pear rootstocks. The application of NO caused a significant increase of the endogenous NO content in plants under NaCl stress. The lowest endogenous NO content was observed in control plants while the highest endogenous NO content was obtained from Pyrodwarf pear rootstocks with 1 mM exogenous NO treatment under 150 mM NaCl stress (Fig. IA).

Stem elongation of Pyrodwarf pear rootstock height

The increment of Pyrodwarf pear rootstocks height significantly reduced by different concentrations of NaCl salinity stress during 7 weeks. Application of NO through root system significantly increased the height of pear rootstocks under NaCl stress conditions compared to plants without exogenous NO treatment under NaCl stress. The highest height of pear rootstocks was observed in plants without NaCl stress with the application of 1 mM exogenous NO while the lowest height of pear rootstocks was recorded under 150 mM NaCl stress and without exogenous NO application (Fig. IB).

RWC and lipid peroxidation of leaves

RWC decreased in Pyrodwarf pear rootstock leaves under NaCl stress, and it further decreased by increasing NaCl concentrations.

Table 1
Effect of exogenous NO treatment at 0, 0.1, 0.5, and 1 mM levels on the content of putrescine (Put), spermidine (Spd), spermine (Spm), the ratio of (Spd+Spm)/Put, and total polyamines (PAs) content in leaves of pyrodwarf pear rootstock leaves under NaCl stress at 0, 50, 100, and 150 mM concentrations

treatment	Put (µmol g^{-1} FW)	Spd (µmol g^{-1} FW)	Spm (µmol g^{-1} FW)	(Spd + Spm)/Put	Total PAs (µmol g^{-1} FW)
NO 0 mM+					
NaCl 0 mM	304.4833 ± 16.19 bc	154.40 ± 5.65c	52.8 ± 0.23o	0.68 ± 0.03bc	511.68 ± 18.67 e
NaCl 50 mM	335.80 ± 26.21abc	178.10 ± 15.47abc	70.25 ± 0.31l	0.76 ± 0.10abc	584.15 ± 10.42bcde
NaCl 100 mM	385.55 ± 11.38ab	160.70 ± 5.88bc	72.95 ± 0.31k	0.61 ± 0.01c	619.20 ± 16.14abc
NaCl 150 mM	404.48 ± 16.20a	187.10± 0.01.9abc	87.15 ± 0.37f	0.68 ± 0.02bc	678.73 ± 15.88a
NO 0.1 mM+					
NaCl 0 mM	278.6 ± 25.94c	183.45 ± 11.11abc	58.65 ± 0.25n	0.88 ± 0.06abc	520.7 ± 32.43de
NaCl 50 mM	322.13 ± 37.56abc	177.40 ± 5.42abc	78 ± 0.34i	0.81 ± 0.07abc	577.53 ± 42.63bcde
NaCl 100 mM	351.25 ± 40.52abc	190.95 ± 11.57ab	80.9 ± 0.34h	0.78 ± 0.06abc	623.1 ± 51.71abc
NaCl 150 mM	375.56 ± 29.68ab	186.10 ± 16.16abc	96.75 ± 0.43e	0.77 ± 0.11abc	658.41 ± 13.09ab
NO 0.5 mM+					
NaCl 0 mM	273.65 ± 16.66c	188.85 ± 7.99ab	63.4 ± 0.28m	0.93 ± 0.07ab	525.9±1640de
NaCl 50 mM	287.76 ± 24.45c	184.30 ± 13.27abc	84.3 ± 2.60g	0.96 ± 0.13ab	556.36 ± 11.01cde
NaCl 100 mM	320.61 ± 24.68abc	196.60 ± 8.31a	87.45 ± 0.37f	0.90 ± 0.10ab	604.66 ± 17.03abcd
NaCl 150 mM	343.78±343.78abc	185.35 ± 5.68bc	104.6 ± 0.46b	0.85 ± 0.06abc	633.73 ± 33.39abc
NO 1 mM+					
NaCl 0 mM	317.43 ± 17.66abc	187.65 ± 7.30abc	74.45 ± 0.31j	0.83 ± 05abc	579.53±19.08bcde
NaCl 50 mM	347.20 ± 0.23abc	181.80 ± 1.61abc	99.05 ± 0.43d	0.81 ± 0.01abc	628.05 ± 1.41abc
NaCl 100 mM	316.36 ± 31.43bc	195.35 ± 7.59a	102.8 ± 0.46c	0.95 ± 0.07bc	614.51 ± 38.56abc
NaCl 150 mM	330.51 ± 33.46abc	192.60 ± 13.91ab	122.9 ± 0.51a	0.98 ± 0.14a	646.01 ± 19.04ab

Different letters indicate significant differences according to Duncan's test (P≤0.05).

Application of NO at 0.1, 0.5, and 1 mM increased the RWC in rootstock leaves under 50, 100, and 150 mM NaCl stress, and no significant difference was observed among exogenous NO levels in plants without NaCl stress (Fig. 2A). The lowest RWC was found in rootstock leaves under 150 mM NaCl stress without NO treatment and application of 1 mM NO did not show a significant difference in plants under 50 mM NaCl compared to the control (Fig. IIA). NaCl stress increased MDA content of Pyrodwarf pear rootstock leaves. The content of MDA is an indicator for membrane lipid peroxidation. The application of NO decreased MDA content of plants under NaCl stress and it was further reduced by increasing the exogenous NO levels. The highest MDA content was observed in plants under 150 mM NaCl without exogenous NO while the lowest MDA content was obtained from the plants treated with 1 mM exogenous NO (Fig. IIB).

Free polyamines contents

As shown in Table I, Putrescine content significantly increased in leaves of Pyrodwarf pear rootstocks under NaCl stress. Exogenous NO decreased Putrescine content in leaves, but its reducing effect was not significant. The highest Putrescine content was observed in 150 mM NaCl treatment without exogenous NO application (Table 1). NaCl stress had no significant effect on Spermidine content in plants and this may be due to the effect of NO on alteration of the ratio of Putrescine, Spermidine and Spermine polyamines under NaCl stress. Exogenous NO treatment significantly increased Spermidine content in

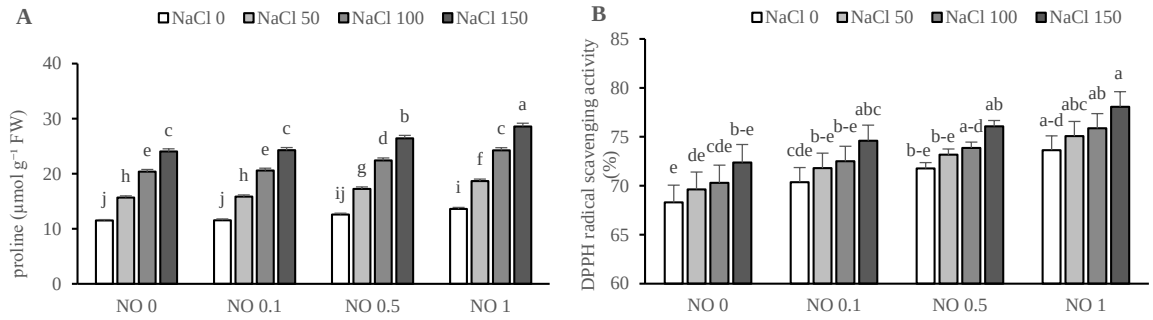

Fig. III. Effect of exogenous NO treatment at 0, 0.1, 0.5, and 1 mM on the content of proline (A) and DPPH radical scavenging activity (B) in leaves of pyrodwarf pear rootstocks under NaCl stress at 0, 50, 100, and 150 mM concentrations; different letters indicate significant differences according to Duncan's test (P ≤ 0.05).

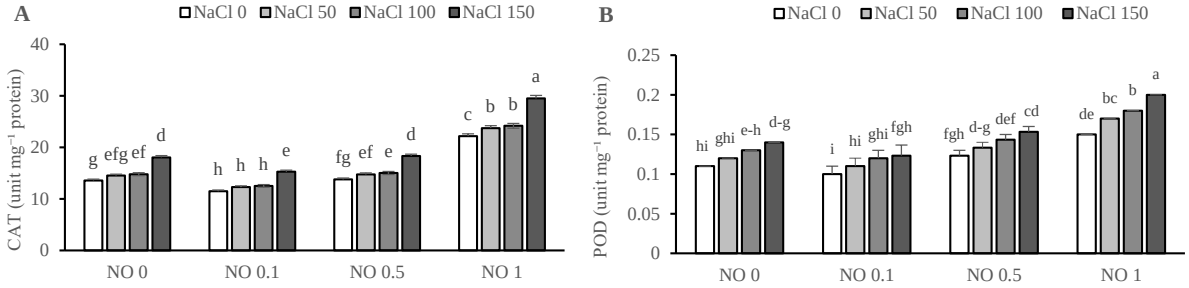

Fig. IV. Effect of exogenous NO treatment at 0, 0.1, 0.5, and 1 mM on the activity of CAT (B), POD (C) enzymes in leaves of pyrodwarf pear rootstocks under NaCl stress at 0, 50, 100, and 150 mM concentrations; different letters indicate significant differences according to Duncan's test (P ≤ 0.05).

leaves of Pyrodwarf pear rootstocks under 100 mM NaCl stress (Table 1). NaCl stress and exogenous NO had a significant effect on the Spermine content in Pyrodwarf pear rootstock leaves. The content of Spermine increased with increasing NaCl concentrations. The application of NO further increased Spermine content in plant leaves. The lowest Spermine content was found in control plants while the highest Spermine content was observed in 1 mM NO treatment under 150 mM NaCl stress (Table 1). The value of (Spermidine + Spermine)/ Putrescine showed no significant differences in Pyrodwarf pear rootstock leaves under NaCl stress. The application of NO significantly increased (Spermidine + Spermine)/ Putrescine value in plant leaves, and the highest (Spermidine + Spermine)/ Putrescine value was obtained in NO-treated plants under 150 mM NaCl stress (Table 1). The content of total polyamines significantly increased in the leaves of the Pyrodwarf pear rootstocks under NaCl stress. Exogenous NO treatment did not show significant difference in the content of total polyamines in pear rootstock leaves. The highest total polyamines content was observed in rootstock leaves under 150 mM NaCl stress without NO treatment, and the lowest polyamines content was shown in control plants (Table 1).

Proline content

As shown in Fig. IIIA, the content of proline significantly increased in leaves of Pyrodwarf pear rootstocks under NaCl stress. NO treatment further increased proline content in plants which was significant. The control plants had the lowest proline content, and the highest proline content was observed in plants treated with 1 mM NO treatment under 150 mM NaCl stress (Fig. IIIA).

The activity of DPPH radical scavenging

DPPH radical scavenging activity, ·OH scavenging capacity, is defined as an antioxidant activity of plant tissues. Exogenous NO significantly induced the scavenging activity of the DPPH radical in Pyrodwarf pear rootstock leaves under NaCl stress. The lowest DPPH scavenging activity was observed in control plants while the

maximum activity of DPPH radical scavenging was recorded in NO-treated plants at 1 mM level under 150 mM NaCl (Fig. IIIB).

The activities of CAT and POD enzymes

As shown in Fig. 4, the activity of CAT and POD enzymes significantly increased in response to NaCl stress and increased wiith increasing NaCl sterss. The application of NO further enhanced CAT and POD activities (Fig. IV). Control plants had the minimum values of CAT and POD enzyme activities. Exogenous NO at 1 mM level significantly increased the activity of CAT and POD enzymes and greatly induced CAT and POD activities in leaves of Pyrodwarf pear rootstocks under 0, 50, 100, and 150 mM NaCl stress (Fig. IV).

Discussion

The application of NO enhanced the content of endogenous NO that can act as signaling molecule or ROS scavenger under stress conditions by improving the activities of antioxidant enzymes (Fan and Liu, 2012; Xu et al., 2010). Arabidopsis mutant decreased the activity of nitric oxide synthase (NOS) that resulted in a reduction in the content of endogenous NO in the plant, and it had more susceptibility under NaCl stress compared with the wild type (Zhao et al., 2007). In this experiment, the application of NO treatment through the root system in Pyrodwarf pear rootstocks led to the enhancement of endogenous NO content in leaves (Fig. IA).

Exogenous NO led to increasing plant tolerance under salt stress (Wu et al., 2011). Treatment of 100 µM NO improved growth of cucumber seedlings under 50 mM NaCl stress and led to an increase in the height of plant under NaCl stress (Fan et al., 2007). Application of NO significantly increased stem length in *Gossypium hirsutum* (Dong et al., 2014), wheat (Hasanuzzaman et al., 2011), rice (Mostofa et al., 2015), and chickpea (*Cicer arietinum*) L. (Ahmad et al., 2016) under NaCl salinity stress. Exogenous NO improved the growth of plants in cucumber (Fan et al., 2007) and citrus (Tanou et al., 2009) under salinity stress. NO can increase cell wall fluidity, cell elongation, and ultimately plant growth (Leshem and Haramaty, 1996). In this experiment, increasing the levels of exogenous NO led to a further increase in the height of the Pyrodwarf pear rootstocks (Fig. IB).

RWC was adversely affected by salinity stress, and the application of NO had a positive effect on the conservation of RWC in plant leaves such as *brassica juncea* (Zeng et al., 2011) and chickpea (Ahmad et al., 2016). The application of SNP significantly increased RWC of leaves, and reduced ion leakage in maize seedling under NaCl stress (Zhang et al., 2006). In this study, application of NO had a positive effect on RWC of Pyrodwarf pear rootstock leaves under NaCl stress (Fig. IIA). The application of NO significantly reduced the damage caused by lipid peroxidation and MDA content in tomato (Manai et al., 2014) and *Brassica juncea* (Zeng et al., 2011) leaves under NaCl stress. In the present study, exogenous NO resulted in the reduction of the MDA, and led to decreased peroxidation of the membrane lipids in Pyrodwarf pear rootstock leaves under NaCl stress (Fig. IIB).

NO regulated the metabolism of polyamines in *Medicago truncatula* leaves, and NO may also remove ROS through polyamines (Filippou et al., 2013). Polyamines are able to maintain the structure and biological function of macromolecules and increase the activity of peroxidase (POD) and catalase (CAT) (Fan et al., 2013). They thereby eliminate ROS, indirectly protect the cell membrane, and promote protein synthesis during stress periods (Fan et al., 2013). Studies have shown that the content of total polyamines were increased in plants under NaCl stress and that exogenous polyamines could feasibly enhance the salinity tolerance of crops (Duan et al., 2008). Putrescine content in cucumber seedlings increased under NaCl stress, and the application of NO reduced the Putrescine content in cucumber seedlings (Fan et al., 2013). Studies demonstrated that Putrescine accumulates in plants under stress; however, an excess of Putrescine resulted in plant damage (Krishnamurthy and Bhagwat, 1989). Putrescine may act as a buffer and osmolyte, which induced an increase in the content of proline and resulted in the preservation of leaf water under stress conditions (Kotakis et al., 2014). Spermine content increased in cucumber seedlings under NaCl stress, and exogenous NO further elevated

Spermine content in plants under NaCl stress (Fan et al., 2013). Generally, enhancement of Spermine and Spermidine contents in plants is favorable for salinity tolerance when they face salinity stress. Salinity caused the accumulation of Putrescine in salinity-sensitive rice cultivars and with little change, in the Spermidine and Spermine content (Krishnamurthy and Bhagwat, 1989). In the present study (Table 1), NaCl stress induced the accumulation of Putrescine, Spermine, and the content of total polyamines, but no significant difference was found in the content of Spermidine and (Spermidine + Spermine)/ Putrescine value under NaCl stress which could be due to the increase in the content of Putrescine and Spermine and also conversion of Putrescine to Spermidine and Spermidine into Spermine. In our experiments, exogenous NO reduced the accumulation of Putrescine and the content of total polyamines under NaCl stress in Pyrodwarf rootstock leaves, on the other hand, it increased the content of Spermine, Spermidine, and the value of (Spermidine + Spermine)/ Putrescine (Table 1). The role of endogenous polyamines in regulating the activity of the plasma membrane H^+-ATPase has been confirmed, and it has been shown that Spermine impressed the proton pump H^+-ATPase whereas Putrescine and Spermidine polyamines had no such an effect (Garufi et al., 2007). NO and polyamines could regulate ROS metabolism (Duan et al., 2008). NO may also act through polyamines in the elimination of ROS (Fan et al., 2013). Polyamines indirectly preserved the cell membrane and improved protein synthesis during the stress (Shen et al., 2000).

Proline is one of the most common osmolytes in plants under stress (Urano et al., 2009) and it increases the tolerance of plants under NaCl stress (Reddy et al., 2015). Proline in plants is synthesized from either glutamate or ornithine (Ashraf and Foolad, 2007). Increasing proline and polyamines accumulation in response to various abiotic stresses may reveal that they participate in ornithine as a common precursor (Alcázar et al., 2010). Application of NO increased proline content in wheat seedlings (Ruan et al., 2004) and *Medicago truncatula* plants (Filippou et al., 2013), as well as tomato plants (Hayat et al., 2012) and cucumber seedlings (Fan et al., 2007) under NaCl stress, which were in agreement with the results of this experiment (Fig. IIIA).

Exogenous NO increased the activity of DPPH radical scavenging and antioxidant enzymes of cucumber hypocotyl and radicle under salt stress (Lin et al., 2012). Exogenous NO protected plants under salinity stress by activating antioxidative enzymes and modifying antioxidant compounds in cucumber (Shi et al., 2007), *Brassica juncea* (Zeng et al., 2011), Citrus aurantium L. (Tanou et al., 2012), and chickpea (Ahmad et al., 2016). In studies on pear (*Pyrus communis*), it was shown that polyamines, at least in part, act by improving the activity of enzymatic and non-enzymatic antioxidants in tolerance to salinity stress (He et al., 2008; Wen et al., 2011). Eexogenous NO induced the activities of ROS scavenging enzymes and decreased ROS accumulation in plants under NaCl stress (Lin et al., 2012; Shi et al., 2007). In this study, the activity of DPPH radical scavenging, CAT and POD enzymes increased under NaCl stress and application of exogenous NO further enhanced the activity of CAT and POD enzymes under NaCl stress (Figs. IIIB and IV).

In this experiment, exogenous NO mitigated the negative effects of NaCl stress through regulating polyamines content in Pyrodwarf pear rootstocks under NaCl stress, which increased Spermine and reduced Putrescine contents (Table I), and led to the induction of antioxidant activities and higher content in proline and lower content of MDA in rootstock leaves under NaCl stress. Also, it is considerable that plants treated with 1 mM NO under 50 mM NaCl stress were not significantly different compared to the control plant, which can be remarkable for stress tolerance under short-term salinity conditions.

Conclusion

The results of this study demonstrated that application of NO through the root system, increased the content of endogenous NO in Pyrodwarf pear rootstock leaves and influenced the ratio of Putrescine, Spermidine, Spermine polyamines and antioxidant capacity. The application of NO increased Spermidine content, and plants with higher Spermine contents resulted

in the induction of antioxidant activity. Exogenous NO ameliorate the adverse effects of NaCl salinity and improved the physiological characteristics of the Pyrodwarf pear rootstocks under NaCl stress.

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