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Sodium Nitroprusside Alleviates Salinity Stress in Pyrodwarf Pear Rootstocks

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ABSTRACT

Nitric oxide (NO) is recognized as a crucial signaling molecule involved in plant defense mechanisms, including salinity stress. The present study investigated the effects of sodium nitroprusside (SNP) application, as a donor of NO, on some morphophysiological and biochemical characteristics of stress tolerance in Pyrodwarf pear rootstocks (*Pyrus communis*) under salinity stress. The experiment was conducted as a factorial design that tested 4 SNP (0, 0.1, 0.5, and 1 mM) × 4 NaCl (0, 50, 100, and 150 mM) levels. Salinity stress reduced leaf count, shoot length, and protein content. However, it increased electrolyte leakage, H2O2, ascorbate content, and leaf concentrations of Na+ and Cl–. SNP application significantly increased leaf count, protein content, leaf chlorophyll and carotenoids. Moreover, it effectively reduced electrolyte leakage (EL), H2O2, and leaf concentrations of Na+ and Cl–. The activity of ascorbate peroxidase (APX), an important antioxidant enzyme, increased in response to NaCl stress, and notably, the application of SNP further enhanced the activity of this enzyme. The results indicated that SNP application reduced NaCl stress to some extent. Despite the fact that no significant interaction was detected in growth indices, SNP mitigated the adverse effects of salinity, primarily through its main effects on growth and its interactive effects on biochemical and antioxidant traits. Overall, the findings highlighted the pivotal role of SNP in enhancing salinity tolerance under short-term stress conditions by modulating physiological and biochemical responses.

Abbreviations: Ascorbate peroxidase (APX), Ascorbate (ASC), Electrolyte leakage (EL), Hydrogen peroxide (H_2O_2), Nitric oxide (NO), Sodium nitroprusside (SNP), Superoxide dismutase (SOD)

Introduction

Salt stress is a major abiotic factor that adversely affects plant growth and development by restricting water and nutrient uptake (Maia et al., 2016; Çelik et al., 2017). In saline soils, the excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions can exert toxic effects on plant cells, disrupting cellular homeostasis (Arif et al., 2020). High salt concentrations lead to osmotic stress and ionic toxicity, triggering a cascade of morphological, physiological, biochemical, and molecular alterations in plants. Critical physiological processes such as photosynthesis and transpiration are especially vulnerable to salt-induced damage (Wani et al., 2013). Salt tolerance varies among pear species. For instance, *Pyrus betulifolia* showed resilience under 100 mM NaCl for five weeks and maintained an 80% survival rate at 150 mM, whereas *P. pyrifolia* exhibited visible damage at just 25 mM

NaCl (Okubo and Sakuratani, 2000). European pear (*Pyrus communis*) demonstrated only slight growth reductions when irrigated with saline water at approximately 5 dS m^{-1}, regardless of genotype, indicating a relatively higher short-term salinity tolerance in *P. communis* rootstocks (Musacchi et al., 2006). Although pear trees are generally cultivated in low-salinity regions, salinity issues can emerge due to specific conditions such as coastal cultivation or the use of saline irrigation or fertigation water (Musacchi et al., 2006). Severe NaCl stress intensifies the generation of reactive oxygen species (ROS), which severely impair normal plant functions by damaging cellular components, including photosynthetic pigments. In response, plants activate an array of antioxidant defense mechanisms. These include both enzymatic antioxidants (e.g. ascorbate peroxidase [APX]) and non-enzymatic antioxidants such as ascorbate (ASC), phenolic compounds, various sugars, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, glycine betaine (GB), and dehydroascorbate (DHA) (Shahzad et al., 2022; He et al., 2008). These antioxidants play a crucial role in scavenging ROS, thereby enhancing plant tolerance to salinity stress and strengthening overall defense responses (Ahmad et al., 2020).

The application of sodium nitroprusside (SNP) has emerged as an effective strategy for alleviating the adverse effects of abiotic stresses in plants. Under saline conditions, SNP serves as a nitric oxide (NO) donor, enhancing plant tolerance mechanisms against salt-induced damage (Hayat et al., 2012). Nitric oxide is a multifunctional signaling molecule involved in plant responses to a range of abiotic stresses, including drought (Nabi et al., 2019) and salinity (Shams et al., 2019). Its synthesis is stimulated by both enzymatic and non-enzymatic antioxidant systems (Nagai et al., 2020). NO plays a central role in regulating key physiological processes, promoting plant adaptation, acclimation, and growth under stressful environments, particularly under high salinity (Choudhary et al., 2023). One of the critical functions of NO is its regulation of compatible solutes such as proline, which helps maintain cellular turgor pressure and improves stress tolerance while supporting normal plant growth (Esim et al., 2024). Additionally, exogenous NO has been shown to enhance photosynthetic efficiency under stress by improving stomatal conductance, increasing CO_2 assimilation, and optimizing overall photosynthetic performance (Farouk et al., 2020; Ferreira et al., 2023). In Malus rootstocks, exogenous NO application promoted the accumulation of compatible solutes such as soluble proteins and reduced malondialdehyde (MDA) levels, thereby facilitating osmotic adjustment and reducing the impact of drought stress (Zhang et al., 2016). Similarly, SNP application in *Malus domestica* rootstocks (‘Fuji’/M9) mitigated the detrimental effects of salt stress, likely through the regulation of biochemical and antioxidant defense mechanisms (Aras et al., 2020). Building upon these findings, the present study investigated the impact of SNP treatment on salinity stress tolerance in Pyrodwarf pear rootstocks (*Pyrus communis*). We hypothesized that exogenous SNP would mitigate NaCl-induced stress through enhancement of antioxidant activity and ion homeostasis. Specifically, the study aimed to evaluate the role of SNP in modulating physiological and biochemical parameters associated with plant defense systems under saline conditions.

Materials and Methods

Plant materials and growth conditions

Pyrodwarf pear rootstocks were propagated via micropropagation and were transplanted into plastic pots (26 cm × 27 cm). Each rootstock measured approximately 30 cm in height. The potting medium consisted of a 1:1 (v/v) mixture of perlite and vermiculite, and plants were cultivated under hydroponic greenhouse conditions. Plants were supplied with Hoagland’s nutrient solution.

Experimental design and treatments

A factorial experiment was arranged in a completely randomized design (CRD), including 4 levels of SNP and 4 levels of NaCl with three replicates per treatment, involving a total of 48 plants. Before carrying out experimental treatments, the plants were allowed a period of establishment to ensure adequate vegetative development, reaching up to an approximate 20 cm increase in rootstock height and the formation of a well-established root system, compared to the initial seedling height. SNP, as an NO molecule donor, was applied to the root system at concentrations of 0, 0.1, 0.5, and 1 mM, dissolved in the nutrient solution. Applications were repeated every two weeks for three times. Then, 48 h after the first SNP application, NaCl was introduced to the root system at concentrations of 0, 50, 100, and 150 mM. Both SNP and salinity treatments were maintained for seven weeks, after which final sampling was conducted.

Growth measurement

The increase in rootstock height was assessed by measuring the difference between the initial height (pre-SNP and NaCl treatment) and the final height (at the end of the seven-week experimental period). This difference was recorded as the increase in rootstock height. Leaf count was recorded as the number of new leaves grown during the seven-week experimental period.

Measurement of electrolyte leakage and protein content

Electrolyte leakage (EL%) is indicative of cell membrane damage, which was assessed using a method outlined by Lutts et al. (1996). The following equation was used:

$$EL\% = \frac{Initial\ Conductivity - Final\ Conductivity}{Initial\ Conductivity} \times 100$$

This equation provides a quantitative measure of electrolyte leakage, reflecting the extent of cellular damage. The total soluble protein (µg g^{-1} FW) of powdered fresh leaves was examined based on Bradford (1976). The absorbance of the extracted samples was measured at a wavelength of 595 nm using a spectrophotometer. Total protein content was quantified and expressed as g 100 g^{-1} FW.

Quantification of photosynthetic pigments

Total chlorophyll (mg g^{-1} FW) and carotenoids (µg g^{-1} FW) were extracted from leaf tissue using 80% acetone according to Lichtenthaler's method (1987). The absorbance by pigments was measured by a UV-visible spectrophotometer (Analytik Jena, Spekol 1500) at 470, 648, and 664nm. After calculation, the total chlorophyll and carotenoids were expressed as mg of each pigment per gram of fresh leaf weight.

Na^+ and Cl^- concentration

At the end of the experiment, samples of dried and powdered leaves were analyzed for the measurement of Na^+ (mg g^{-1} DW) and Cl^- (mg g^{-1} DW). The samples were extracted with dilute nitric acid. The Na^+ concentration of the extracts was determined using a flame photometer.

The samples were first digested with a mixture of 5 mL of 2-nitrogen hydrochloric acid and nitric acid, followed by boiling and becoming dissolved in water, and finally filtered. Subsequently, the final volume was reduced to 50 mL (Gao et al., 2016). The Cl^- concentration was obtained by titration with silver nitrate (Chapman and Pratt, 1961).

Determination of H_2O_2 and ASC content

H_2O_2 content (µmol g^{-1} FW) was determined following a method described by Velikova et al. (2000). Briefly, 0.2 g of leaf tissue was homogenized in liquid nitrogen and trichloroacetic acid. Subsequently, 0.5 mL of the supernatant was combined with 1 mL of 1 M potassium iodide and 0.5 mL of 10 mM potassium phosphate buffer (pH = 7.5). The absorbance of the resultant solution was measured at 390 nm. H_2O_2 concentrations were determined using a standard curve generated with known H_2O_2 concentrations.

The ASC content (mmol g^{-1} FW) was determined using a method described by Hodges et al. (1996). Samples (0.3 g) were homogenized in 2 mL of 5% (w/v) m-phosphoric acid. The homogenates were centrifuged at 12,000 × g for 20 min at 4 °C, and the resultant supernatant (100 µL) was mixed with 500 µL of 150 mM KH_2PO_4 buffer (pH = 7.4) that contained 5 mM EDTA. To achieve color development, the procedure involved sequential additions of 400 µL trichloroacetic acid (10% w/v), 400 µL o-phosphoric acid (44% v/v), 400 µL of o-dipyridyl dissolved in ethanol (70% v/v), and 200 µL $FeCl_3$ (0.03% w/v). The reaction mixtures were incubated at 40 °C for 1 h, and the absorbance was measured at 525 nm.

Measurement of SOD and APX activities

The leaves were homogenized in 0.1 M potassium phosphate buffer (pH = 7) containing insoluble PVP and EDTA. The homogenate was filtered and then centrifuged for 10 min at 10,000 g at 4 °C. The supernatant was used for the determination of the catalytic activity of the enzymes. The SOD activity (unit mg^{-1} protein) was assayed according to a method described by Beyer Jr and Fridovich (1987). One unit of activity was defined as the amount of enzyme necessary to cause a 50% inhibition of the reduction of nitroblue tetrazolium (NBT) at 560 nm. The APX activity (unit mg^{-1} protein) was measured according to Nakano and Asada (Nakano and Asada, 1981), following a decrease in the oxidation of ascorbate by adding H_2O_2 at 290 nm.

Statistical analysis

After data collection, the analysis of variance was performed. To test significant differences among treatments, a general linear model (GLM) was applied. The significance of the differences were tested using Duncan's New Multiple Range Test (DMRT) ($P \leq 0.05$) and considered to be statistically significant in that range. All statistical analyses were carried out by SPSS, version 22, for Windows (IBM SPSS Statistics release 22.0.0, 2013, SPSS Inc., Chicago, IL, USA).

Results

Plant growth

Leaf count of pear rootstocks declined significantly with increasing NaCl concentrations, with the lowest values recorded at 150 mM compared to the control (Table 1 and Fig. 1A). Independently, SNP application significantly increased leaf count, and the maximum value was observed at 1 mM SNP under non-saline conditions. These results indicated that both salinity and SNP had significant main effects on leaf count. Leaf count decreased by 80% at 150 mM NaCl compared to the control, while SNP restored it by 40%.

Table 1. Analysis of variance on the effect of different levels of NaCl and SNP treatment on leaf count, shoot length, EL%, total protein and photosynthetic pigments in the leaves of Pyrodwarf pear rootstocks.

		Mean of squares							
S.O.V	**DF**	**Leaf count**	**Shoot length**	**EL%**	**Total protein**	**Chl *a***	**Chl *b***	**Total Chl**	**Carotenoid**
NaCl	3	177.97**	451.24**	633.93**	80.61**	80.61**	46.74**	234.59**	43.73**
SNP	3	15.52**	115.69**	140.40**	11.20**	11.20**	6.53**	32.97**	6.67**
NaCl×SNP	9	0.67	1.35	16.39**	2.20*	2.20	1.30*	6.43*	1.34
Error	32	1.25	10.81	5.41	1.03	1.03	0.52	2.82	0.78
CV.(%)		15.47	26.00	9.06	5.64	1.57	5.24	5.46	6.41

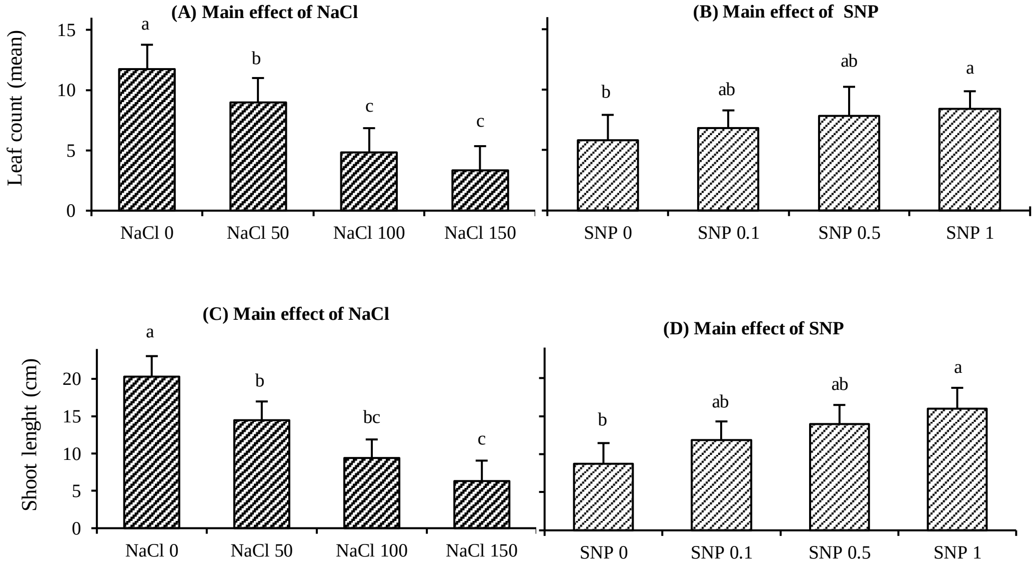


Fig. 1. Effects of NaCl stress (0, 50, 100, and 150 mM) and SNP application (0, 0.1, and 0.5 mM) on (**A**, **B**) leaf count and (**C**, **D**) shoot length in Pyrodwarf pear rootstocks. Different letters indicate significant differences among treatment means according to Duncan's test ($P \leq 0.05$).

The shoot length of pear rootstocks decreased significantly with increasing NaCl concentrations, showing an 85% reduction at 150 mM compared to the control (Table 1 and Fig. 1B). Independently, SNP application to the root system exerted a significant positive effect, with the highest shoot length recorded under non-saline conditions at 1 mM SNP. In contrast, the lowest shoot length was observed under 150 mM NaCl without SNP application. These results confirm significant main effects of both salinity and SNP on shoot length.

Electrolyte leakage and protein content of leaves

Salinity stress markedly increased electrolyte leakage in the leaves of Pyrodwarf rootstocks. However, the SNP treatment significantly reduced electrolyte leakage and alleviated membrane injury in leaf cells. The highest electrolyte leakage was recorded in rootstocks exposed to 150 mM NaCl, whereas the lowest leakage was observed in non-stressed plants treated with 1 mM NO (Fig. 2A and Table 1).

Furthermore, increasing NaCl concentrations (50, 100, and 150 mM) significantly reduced protein content in Pyrodwarf rootstocks. Nevertheless, the exogenous application of SNP, particularly at 1 mM, effectively mitigated this decline and resulted in a significant increase in protein content in response to salinity stress (Fig. 2B).

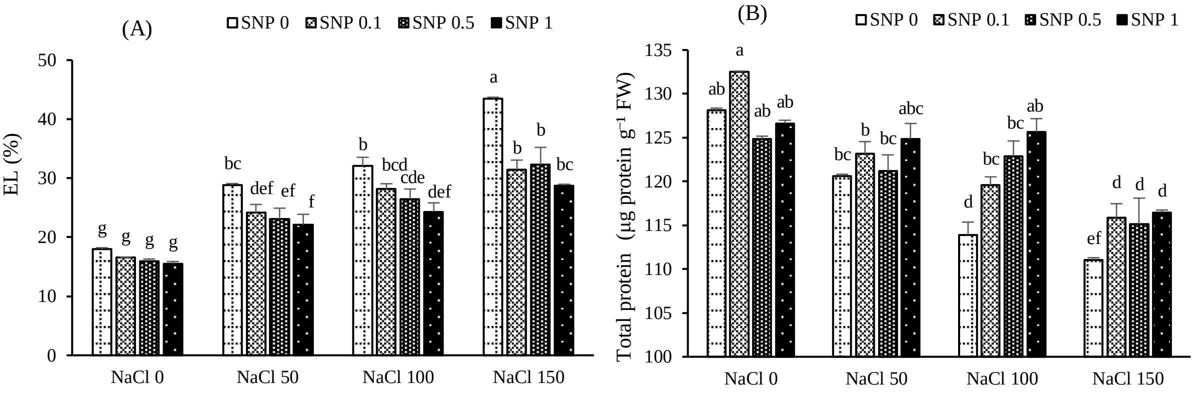


Fig. 2. Effect of SNP treatment at levels 0, 0.1, 0.5 and 1 mM on the (**A**) EL% and (**B**) total protein in the leaves of Pyrodwarf pear rootstocks under NaCl stress at concentrations 0, 50, 100 and 150 mM. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

Photosynthetic pigments

The analysis of variance (Table 1) indicated that salinity stress had a significant effect on chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents in Pyrodwarf pear rootstocks. As the NaCl concentration increased from 0 to 50 mM, significant increases were observed in chlorophyll *a* (Fig. 3A), chlorophyll *b* (Fig. 3B), total chlorophyll (Fig. 3C), and carotenoids (Fig. 3D). However, further increases in the NaCl concentration to 100 and 150 mM led to a marked decline in these pigment levels. Applying SNP exogenously at concentrations of 0.1, 0.5, and 1 mM significantly enhanced chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents in Pyrodwarf leaves compared to plants that did not receive the NO treatment. Notably, 1 mM SNP significantly improved chlorophyll *b* content under 50 and 100 mM NaCl stress, but no significant effect occurred in response to the 150 mM NaCl stress (Fig. 3).

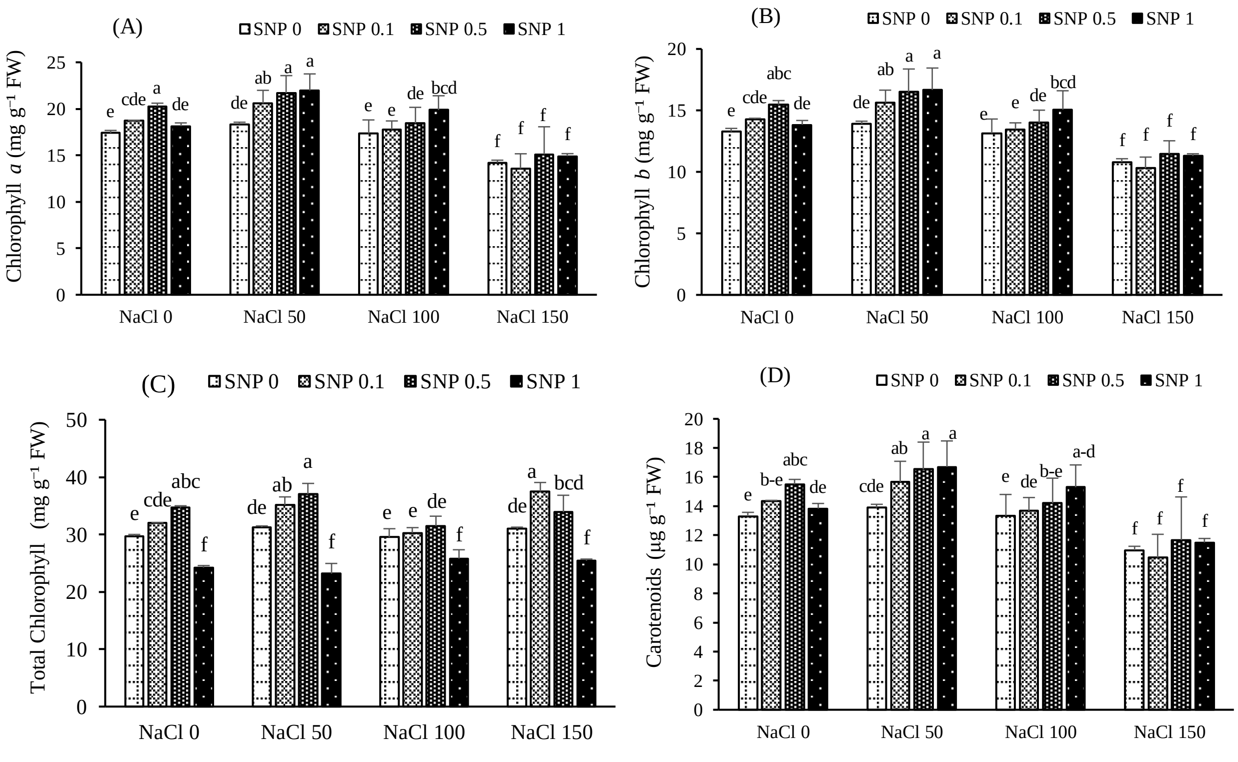


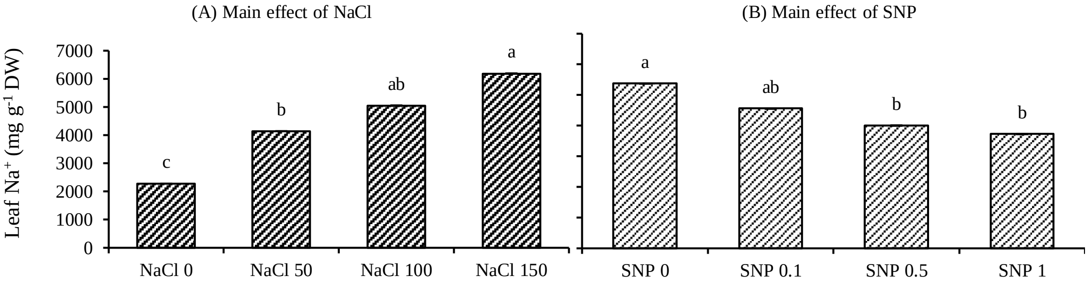
Fig. 3. Effect of SNP treatment at levels 0, 0.1, 0.5 and 1 mM on Photosynthetic pigments including (**A**) chlorophyll *a*, (**B**) chlorophyll *b*, (**C**) total chlorophyll, and (**D**) carotenoids in the leaves of Pyrodwarf pear rootstocks under NaCl stress at concentrations 0, 50, 100 and 150 mM. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

Leaf Na^+ and Cl^- concentrations

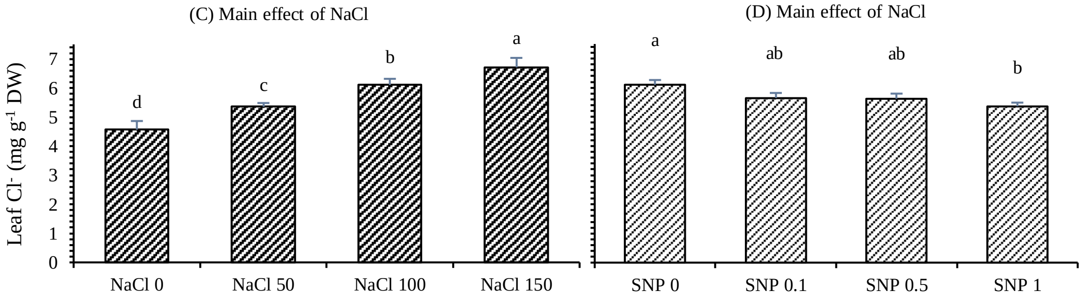
Analysis of variance indicated that both salinity and SNP treatments significantly affected leaf Na^+ and $Cl^–$ concentrations (Table 2). NaCl-induced salinity stress markedly increased Na^+ and $Cl^–$ accumulation in Pyrodwarf pear rootstocks (Fig. 4), with leaf Na^+ content positively correlated with NaCl concentration. Exogenous application of SNP significantly alleviated this ionic accumulation in NaCl-stressed plants. Notably, rootstocks exposed to 50 mM NaCl and treated with 1 mM SNP showed leaf Na^+ and $Cl^–$ levels comparable to non-stressed controls. The highest Na^+ and $Cl^–$ concentrations were recorded in plants treated with 150 mM NaCl without SNP, whereas the lowest occurred in control plants (Fig. 4A and B). Although the interaction between salinity and SNP was not significant, SNP effectively mitigated salinity-induced Na^+ and $Cl^–$ accumulation.

Table 2. Analysis of variance the effect of different levels of NaCl and SNP treatment on Na^+, Cl^-, H_2O_2, ASC content, and SOD and APX enzyme activity in the leaves of Pyrodwarf pear rootstocks.

	Mean of squares						
S.O.V	**DF**	**Na^+**	**Cl^-**	**H_2O_2**	**ASC**	**SOD**	**APX**
NaCl	3	32785052.08**	10.34**	0.25**	1950.03 **	300649.85**	0.53**
SNP	3	6348107.64**	1.13**	0.01**	265.87 **	14445.65**	0.84**
NaCl×SNP	9	675885.42	0.19	0.00	32.79 *	26.69	0.01**
Error	32	495260.42	0.11	0.00	36.63	2179.69	0.00
CV.(%)		15.94	5.87	4.05	11.19	2.52	3.58


(A) Main effect of NaCl

(B) Main effect of SNP


(C) Main effect of NaCl

(D) Main effect of NaCl

Fig. 4. Effects of NaCl stress (0, 50, 100, and 150 mM) and SNP application (0, 0.1, and 0.5 mM) on (**A**, **B**) Na^+ and (**C**, **D**) $Cl^–$ concentrations in Pyrodwarf pear rootstocks. Different letters indicate significant differences among treatment means according to Duncan's test ($P \leq 0.05$).

H_2O_2 and ASC contents

The analysis of variance (Table 2) indicated that both NaCl salinity and SNP treatments had significant effects on leaf H_2O_2 content. NaCl stress markedly elevated H_2O_2 levels in pear leaves. However, SNP application at 0.5 and 1 mM concentrations significantly mitigated this increase, resulting in a reduced H_2O_2 content in salt-stressed plants (Fig. 5A).

The ASC content decreased in response to NaCl concentrations of 100 and 150 mM. Exogenous SNP at 0.5 and 1 mM levels improved the ASC content of pear rootstocks despite using 100 mM NaCl. The highest ASC content was observed in plants treated with 1 mM SNP without NaCl stress, and the lowest ASC content was observed in plants treated with 150 mM NaCl (Fig. 5B).

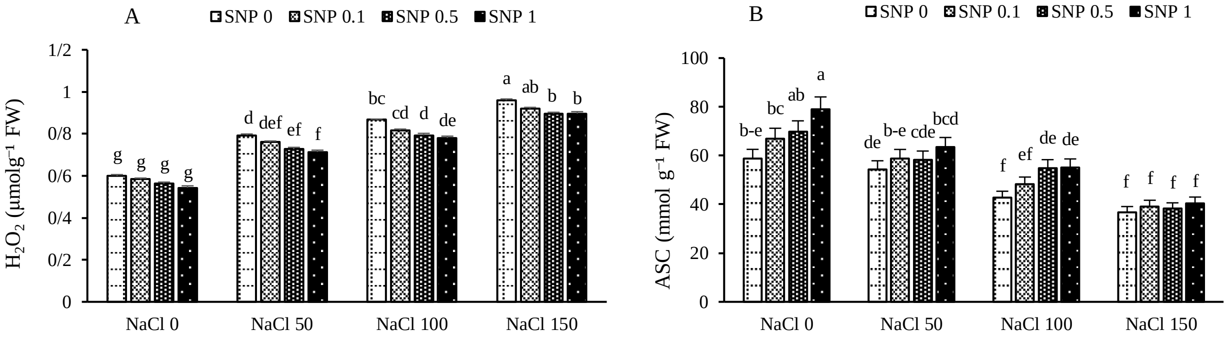


Fig. 5. Effect of SNP treatment at levels 0, 0.1, 0.5 and 1 mM on the content of (**A**) H_2O_2 and (**B**) ASC in leaves of Pyrodwarf pear rootstocks under NaCl stress at concentrations 0, 50, 100, and 150 mM. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

SOD and APX enzyme activities

The analysis of variance (Table 2) demonstrated that salinity stress significantly enhanced SOD activity in pear rootstocks. The lowest SOD activity was observed in control plants (Fig. 6A). APX enzyme activity was also significantly enhanced by NaCl-induced salinity stress on the rootstocks, with the enzyme activity increasing in response to higher NaCl concentrations. Moreover, the SNP application at 0.5 and 1 mM further stimulated APX activity under saline conditions (Fig. 6B).

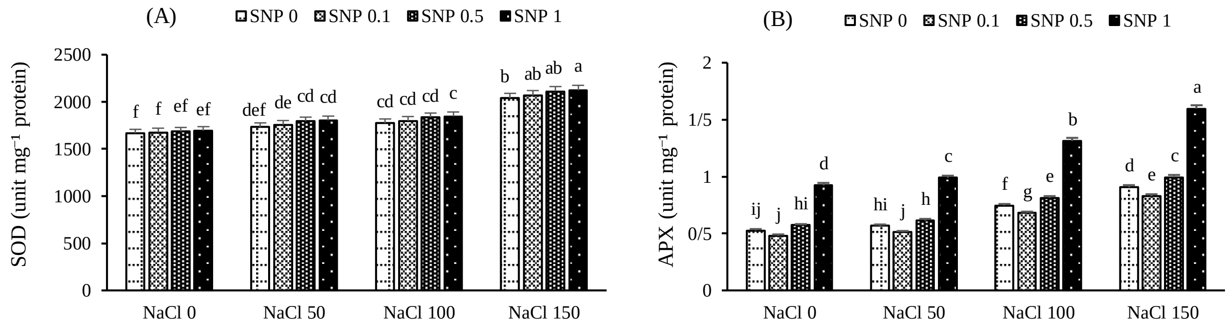


Fig. 6. Effect of SNP treatment at levels 0, 0.1, 0.5, and 1 mM on the activity of the enzymes (**A**) SOD and (**B**) APX in the leaves of Pyrodwarf pear rootstock under NaCl stress at concentrations of 0, 50, 100, and 150 mM. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

Correlation coefficients

Correlation analysis revealed that EL, as an indicator of membrane damage under salt stress, correlated positively with leaf Na^+ and Cl^- contents, while showing a negative correlation with growth parameters, ASC content and photosynthetic pigment levels. Additionally, significant positive correlations were observed among Na^+, Cl^-, EL, H_2O_2, and also among the activities of antioxidant enzymes SOD and APX, suggesting that these traits were interrelated in this research and may play a crucial role in salt stress tolerance mechanisms in plant cells (Table 3).

Table 3. Correlation Analysis of among EL, enzymes (SOD and APX), H_2O_2, ASC, Na^+, Cl^-, protein, Chlorophyll (*a*, *b* and total) and carotenoids.

Indices	**EL**	**SOD**	**APX**	**H_2O_2**	**ASC**	**Na**	**Cl**	**protein**	**Chl *a***	**Chl *b***	**Chl total**	**Car**
EL	1	.672**	.282	.840**	-.773**	.886**	.886**	-.607**	-.558**	-.575**	-.566**	-.534**
SOD	.672**	1	.640**	.830**	-.795**	.685**	.737**	-.643**	-.710**	-.715**	-.712**	-.692**
APX	.282	.640**	1	.427**	-.314*	.252	.347*	-.643**	-.299*	-.311*	-.304*	-.276
H_2O_2	.840**	.830**	.427**	1	-.813**	.836**	.841**	-.583**	-.536**	-.554**	-.544**	-.514**
ASC	-.773**	-.795**	-.314*	-.813**	1	-.843**	-.867**	.492**	.739**	.743**	.742**	.746**
Na	.886**	.685**	.252	.836**	-.843**	1	.866**	-.509**	-.596**	-.605**	-.601**	-.589**
Cl	.886**	.737**	.347*	.841**	-.867**	.866**	1	-.631**	-.623**	-.634**	-.629**	-.606**
protein	-.607**	-.643**	-.643**	-.583**	.492**	-.509**	-.631**	1	.307*	.327*	.316*	.269
Chl *a*	-.558**	-.710**	-.299*	-.536**	.739**	-.596**	-.623**	.307*	1	.999**	1.000**	.992**
Chl *b*	-.575**	-.715**	-.311*	-.554**	.743**	-.605**	-.634**	.327*	.999**	1	1.000**	.989**
Chl total	-.566**	-.712**	-.304*	-.544**	.742**	-.601**	-.629**	.316*	1.000**	1.000**	1	.991**
Car	-.534**	-.692**	-.276	-.514**	.746**	-.589**	-.606**	.269	.992**	.989**	.991**	1

Correlation is significant at the 0.05 level, and ** correlation is significant at the 0.01 level.

Discussion

Studies have demonstrated that NaCl-induced salinity stress reduced plant growth and leaf count among various species, including *Pyrus communis* (Javadisaber et al., 2024), *Pyrus pyrifolia*, and *Pyrus betulifolia* rootstocks (Okubo and Sakuratani, 2000). Pre-treatment with SNP has reportedly alleviated these adverse effects on citrus (Tanou et al., 2009; Tanou et al., 2012; Khoshbakht et al., 2018) and pistachio rootstocks (Mirabi et al., 2024). Similarly, SNP application significantly enhanced relative shoot length and leaf area in Myrobalan (*Prunus cerasifera*) 29C rootstocks under water deficit conditions (Bolat et al., 2022) and mitigated growth inhibition in apple seedlings exposed to salt stress (Aras et al., 2020). The beneficial effects of nitric oxide (NO) on plant growth are primarily attributed to its role in enhancing cell wall flexibility and promoting cell elongation, thereby stimulating overall plant development (Leshem and Haramaty, 1996). Consistent with these findings, exogenous application of SNP at concentrations up to 1 mM significantly increased shoot height and leaf count in Pyrodwarf pear rootstocks under saline conditions (Fig. 1). Although the interaction between salinity and SNP was not statistically significant, these results indicate that the main effects of SNP can promote growth traits under saline environments.

The observed reduction in electrolyte leakage may be attributed to the ability of SNP to preserve membrane integrity under saline conditions, which was mediated through the activation of antioxidant defense systems and improved ionic homeostasis (Liu et al., 2014). In this regard, Khoshbakht et al. (2018) reported that SNP application reduced membrane permeability and lipid peroxidation in citrus, effectively preventing electrolyte leakage. Furthermore, Aras et al. (2020) confirmed that SNP treatment decreased plasma membrane permeability and electrolyte leakage, thus maintaining membrane stability and functionality under salt stress and alleviating salt-induced cellular damage. These results are in agreement with our experimental findings (Fig. 2A).

Salinity stress is known to induce protein oxidation, leading to a consequent decline in protein content (Remorini et al., 2009; Tanou et al., 2009). However, previous studies have demonstrated that NO effectively mitigates protein oxidation under stress conditions (Tanou et al., 2009; Khoshbakht et al., 2018; Aras et al., 2020). In agreement with these reports, the present study showed that SNP significantly preserved protein content and counteracted salinity-induced declines in protein levels in pear rootstocks (Fig. 2B).

Salt stress has been widely reported to adversely affect chlorophyll content, thereby impairing photosynthetic efficiency in pear (Zhang et al., 2024). Bolat et al. (2022) reported that NO application significantly increased chlorophyll levels in Myrobalan rootstocks subjected to water deficit. Similarly, SNP treatment improved chlorophyll content in pistachio rootstocks under NaCl-induced salinity (Mirabi et al., 2024) and elevated chlorophyll content in salt-stressed apple seedlings (Aras et al., 2020), thus confirming the present findings (Fig. 3).

The excessive accumulation of Na^+ and $Cl^–$ is recognized as a primary cause of NaCl stress in plants, triggering a series of morphological, physiological, and biochemical disruptions (Dichala et al., 2021; El-Mahdy et al., 2022). Exogenous SNP significantly reduced Na^+ and Cl^- concentrations in citrus leaves (Khoshbakht et al., 2018), which our findings confirmed, thus demonstrating that pear rootstocks treated with 1 mM SNP under 50 mM NaCl stress exhibited Na^+ and Cl^- levels comparable to those of non-stressed control plants (Fig. 4). Moreover, exogenous SNP reportedly enhanced salinity tolerance by regulating Na^+ homeostasis with other essential nutrients, thereby limiting Na^+ accumulation under saline conditions (Zhao et al., 2004; Zhang et al., 2006).

SNP reduces oxidative damage via APX upregulation, salinity stress potentially triggers an excessive production of H_2O_2, resulting in oxidative damage and impaired cellular functions in plants (Akram et al., 2020). Enhanced activities of key antioxidant enzymes, such as SOD and APX, are central to the detoxification of H_2O_2 and are considered crucial for maintaining redox homeostasis under salt stress (Javadisaber et al., 2023; Aras et al., 2020). NO reportedly modulated the metabolism of reactive oxygen species by promoting the dismutation of $O_2\bullet^-$ to H_2O_2, thus enhancing the activity of antioxidant enzymes (Beligni et al., 2002; Verma and Mishra, 2005).

Previous studies indicated that NaCl stress reduces ASC content in citrus fruits, whereas SNP application restores ASC levels under salinity conditions (Tanou et al., 2009). Similarly, in tomato plants, SNP treatments enhanced salinity tolerance by stimulating APX enzyme activity and increasing leaf ASC content (Manai et al., 2014). These findings are consistent with the results of the present study (Figs. 5 and 6). According to Asada (1999), where leaves are exposed to high salinity levels, the ASC content may reflect elevated APX activity involved in ROS detoxification. The concurrent increase in ASC content under severe salinity stress, along with rising H_2O_2 levels, likely serves to sustain APX activity and preserves cellular redox homeostasis. Exogenous applications of SNP have reportedly boosted the antioxidant defense system, thereby reducing membrane permeability and mitigating oxidative stress in various plant species (Bhardwaj et al., 2021; Mariyam et al., 2023; Kaya et al., 2023), including citrus (Tanou, et al., 2012; Khoshbakht et al., 2018).

In the present study, salinity stress significantly upregulated SOD and APX activities in Pyrodwarf pear rootstocks, suggesting an adaptive antioxidant response. Moreover, SNP supplementation further enhanced APX activity and markedly reduced H_2O_2 accumulation (Fig. 6), indicating its role in the antioxidative defense machinery. Notably, plants treated with 1 mM SNP under 50 mM NaCl stress exhibited no significant differences in oxidative stress parameters compared to control plants, highlighting the influence of SNP in conferring short-term salinity tolerance. The application of SNP offers a cost-effective strategy for mitigating salinity stress, and by maintaining up to 90% of plant growth under 50 mM NaCl stress, SNP could substantially reduce economic losses in saline-prone orchards. However, this study was limited to short-term stress in greenhouse conditions; thus, Long-term field trials are necessary.

Conclusion

The present study demonstrated that salinity stress markedly inhibited the growth of Pyrodwarf pear rootstocks. Salinity stress increased H_2O_2 levels, indicating enhanced oxidative damage, while concurrently stimulating the activities of key antioxidant enzymes. Notably, exogenous SNP effectively alleviated the detrimental impacts of salinity by improving growth and modulating biochemical responses. Specifically, the SNP treatment enhanced the ASC content and the photosynthetic pigment content while reducing oxidative stress. SNP-treated plants maintained 90% growth under 50 mM NaCl These findings suggest that SNP application via the root system could serve as a practical strategy to improve salt tolerance in pear rootstocks, particularly under conditions of limited freshwater availability. Thus, SNP treatment holds potential for optimizing fresh water resources for short-term irrigation in pear cultivation. Future research should investigate SNP effectiveness across different rootstock genotypes and over longer periods.

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Author Contributions

Conceptualization, LN, FZN and MY; methodology, FZN and MY; software, FZN and MY; validation, LN, FZN and MY; formal analysis, FZN and MY; investigation, MY; resources, MY; data curation, MY; writing—original draft preparation, MY; writing—review and editing, MY; visualization, MY; supervision, LN and FZN; project administration, LN; funding acquisition, LN and MY. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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