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Original article

Foliar application of sodium nitroprusside and salicylic acid alleviates the adverse effects of alkaline soil on roses

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Summary

The purpose of this study was to investigate the effects of exogenous sodium nitroprusside (SNP), salicylic acid (SA) and their combination on some growth characteristics and physiological parameters in *Rosa hybrida* 'Beverly Watson' under alkalinity conditions. This experiment was conducted as a factorial experiment based on the completely randomized design with two factors in three replications and two pots in each replication. The factors were 4 concentrations of SNP (0, 0.5, 1 and 2 mM) and 5 concentrations of SA (0, 0.25, 0.5, 1 and 2 mM) as foliar application. In order to create alkaline soil, sodium bicarbonate was applied to the soil of pots until to be obtained pH = 8. The results showed that 2 mM SA caused increasing in the activity of antioxidant enzymes, and 0.5, 1, and 2 mM SNP has positive effects on these factors. In this study, both SNP and SA increased growth characteristics such as fresh and dry weight of flowers and longevity of flowers on the shrubs and decreased alkaline stress, which may be a result of their role in a significant increase in activation of catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX). The application of SNP has a positive effect on proline content, but SA does not have any effect on it. As well, it was observed that they had synergistic effects in CAT activity, electrolyte leakage, and MDA content. Thus, the combination of SNP and SA can be a new indicator of a protective mechanism to lower lipid peroxidation, electrolyte leakage, and facilitate membrane transport to detoxify reactive oxygen species (ROS).

Keywords
alkalinity, ascorbate peroxidase, catalase, guaiacol peroxidase, nitric oxide, *Rosa hybrida*

Abbreviations
SNP: Sodium nitroprusside; SA: Salicylic acid; NO: Nitric oxide; ROS: Reactive oxygen species; CAT: Catalase; APX: Ascorbate peroxidase; GPX: Guaiacol peroxidase; O-M: Organic matter; SAR: Sodium absorption ratio

Significance of this study

What is already known on this subject?
- Rose is a plant sensitive to alkaline soils. Since this plant is grown in large quantities in landscape, alkalinity will cause a decrease in the quality and performance of it.

What are the new findings?
- Sodium nitroprusside combined with salicylic acid could effectively alleviate the injury induced by alkaline soil during the developmental stages of the rose plants.

What is the expected impact on horticulture?
- Since NO and SA participate in the regulation of some physiological processes and plant resistance to biotic and abiotic stresses, they can be applied in horticultural plants, most of which are sensitive to stress.

Introduction

Plants are frequently affected by abiotic stresses, including drought, salinity, alkalinity, and extreme temperatures, which thereby negatively affect their growth and productivity. Worldwide, salt stress affects 831 million hectares, 434 million of which also suffer from sodic alkaline conditions (Gong et al., 2014). Alkalinity is generated by HCO_3^- and CO_3^-, the significant alkalis that expose a buffer capacity to water and result in an increase in pH of solution, caused by the formation of insoluble forms of phosphorus (P) and micronutrients. Chlorosis in young leaves is often shown on plants irrigated with water of high alkalinity, which may also prevent the growth of sensitive plants through alleviated root growth, nutrient uptake and utilization (Cartmill et al., 2008).

Alkaline soils, which are very common in semiarid and arid climates, cover more than 25% of the earth's surface (López-Bucio et al., 2000). In Iran, over 20 percent of the land surface or a total of about 20 million ha, suffers from a combination of salinity and alkalinity. Such soils will have a pH of 8 to 9 or even as high as 10 (Roozitalab et al., 2018). In addition climate, soil characteristics play a remarkable role in the survival and appearance of plants (Caplan and Yeakley, 2006). Soil reaction (pH), in particular, can be considered a key variable due to its effects on many other soil factors affecting plant growth. In fact, microorganism activity as well as nutrients solubility and availability are some of the most important processes that depend on pH (Lončarić et al., 2008). In alkaline soils, although the availability of most macronutrients is increased, phosphorus and micronutrient availability is generally reduced and their lower levels can harmfully affect plant growth. Particularly, many plant traits such as height, lateral spread, biomass, flower size and number, etc., are influenced by pH (Jiang et al., 2017).

Like NaCl and sodic alkaline stress, alkaline stress causes an imbalance in ionic homeostasis owing to excessive Na^+ accumulation (Gong et al., 2014). Alkaline stress decreased plant growth. Growth reduction under stress conditions has been shown by many researchers (Izan et al., 2015; Bai et al., 2015). The reduction in plant growth indicators in stress conditions seems to be the result of disturbed plant water relations, in particular the turgid potential, also decrease in the tissue water contents, cell elongation and damage to cell division (Ghasemi et al., 2016).

Abiotic stress conditions such as alkaline stress cause the accumulation of Reactive Oxygen Species (ROS) such as superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2). High levels of ROS lead in plants to oxidative damage in lipids, proteins, and nucleic acids (Joseph et al., 2010). The concentrations of ROS are controlled by antioxidant defense systems comprising enzymes such as the catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), etc. These enzymes increase scavenging accumulated ROS. The increase of antioxidant enzyme activity can be supposed as an important mechanism in the cellular defense strategy against oxidative stress. CAT, as the key enzyme in the removal of toxic H_2O_2, GPX and APX, also plays critical roles in removing H_2O_2 and prevent increasing the concentration of H_2O_2 (Shi et al., 2006). Also studies have shown that some molecules, such as calcium, hydrogen peroxide, abscisic acid, jasmonic acid, ethylene, salicylic acid, and nitric oxide, are signal transducers or messengers (Simaei et al., 2011).

Proline as an amino acid is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. There are many reports about increasing of proline under environmental stress conditions in plants. For example, in basil plants subjected to salt stress, the concentration of proline was increased in the leaves (Delavari et al., 2010). Proline via osmotic control, avoiding enzymes destruction and removal of hydroxyl radicals, increased the tolerance of the plants against stresses (Kuznetsov and Shevykova, 1999).

Nitric oxide (NO) is a free radical, highly diffusible gaseous molecule that involved in various plant growth and developmental processes, including germination, metabolism, signal transport, flowering and senescence. Besides, evidence indicates that NO is an important endogenous signal molecule that plays a critical role in plant disease resistance, hormone responses, homeostasis, cell death, and stress tolerance (Mansouri, 2012; Gong et al., 2014). Also, NO plays a vital role in ROS scavenging. Exogenous NO can increase antioxidant levels under various stresses by activating antioxidant defense systems, which can play critical roles in plants' tolerance to stress (Aftab et al., 2012).

Salicylic acid (SA) (2-hydroxybenzoic acid) as an essential signal molecule affects in several physiological processes and responses to environmental stress in plants. Exogenous application of SA may direct and indirect affect a range of various processes in plants, including seed germination, stomata closure, ion uptake, and transport, membrane permeability, photosynthetic and growth rate (Yildirim et al., 2008). Also, SA participates in the regulation of physiological processes and plant resistance to biotic and abiotic stress. Furthermore, it is indicated that SA can trigger nitric oxide synthesis in *Arabidopsis* seedlings (Kong et al., 2014). Beyond, SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, production of glycinebetaine (GB), antioxidant defense system, and plant-water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Miura and Tada, 2014; Iqbal et al., 2015).

Roses belong to the family Rosaceae, and are best known as ornamental plants grown for their flowers in the garden, cut flowers and sometimes indoors. Some are used as landscape plants. Since roses are sensitive plants to alkaline stress and these plants are grown in large amounts in landscape, this stress will cause a decrease in the quality and performance of this product. As mentioned above, the soil in Iran is calcareous and contains a diverse range of carbonate minerals. The continuous use of these soils and excessive soil erosion reduces the growth and yield of plants. Under the conditions that both soil and water pH is generally high in Iran as a result of calcareous soil, it is imperative to adopt measures and practices to alleviate the adverse effects of alkalinity on plants. So, the experiment aimed was to study the effects of the application of sodium nitroprusside (NO donor) and salicylic acid on growth characteristics, antioxidant enzymes activity and other defense systems in alkaline soil conditions.

Materials and methods

Plant materials and experiment conditions

In this research, the cuttings of roses (*Rosa hybrida* L.) cv. Beverly Watson were first planted in a sand bed for two months to bear roots. Well-rooted cuttings were transferred to separate pots and were kept there for one month to fully develop new plants and then pruned to 50 cm height. Plastic pots with a diameter of 20 cm and a height of 16 cm were used. The soil used in the pots was composed of garden soil and sand (3:1 v/v). After full establishment of plants, sodium bicarbonate ($NaHCO_3$) (solution containing 100 mM $NaHCO_3$) was applied in three stages concurrent with irrigation of the plants until to obtain pH = 8. In order to analyze the soil of the pots, the soil sample was sent to the soil analysis laboratory in Urmia University's Soil Science Department. The results are presented in Table 1. There isn't any fertilization during experiment. The greenhouse temperature was 28–30/20–23°C day/night with a relative humidity of 60 ± 5%. The plants were irrigated three times a week. After alkaline soil ensures, different concentrations of SNP, as a nitric oxide donor and salicylic acid applied as foliar spray. These treatments were applied weekly for ten weeks.

This experiment was conducted as a factorial trial based on completely randomized design with two factors: four

Table 1. Results of soil analysis.

Texture	Silt (%)	Sand (%)	Clay (%)	pH	EC (ds m^{-1})	O-M (%)	Cl^- (meq L^{-1})	$CaCO_3$ (meq L^{-1})	HCO_3^- (meq L^{-1})	SAR (meq L^{-1})
Sandy clay loamy/ Sandy loamy	20	52	28	8	3.16	2.2	5	22	5.2	22

O-M: Organic matter; SAR: Sodium absorption ratio.

concentrations of SNP (Sigma-Aldrich) (0, 0.5, 1 and 2 mM) and five concentrations of SA (Applichem) (0, 0.25, 0.5, 1 and 2 mM) as foliar application in three replications. Each replication included two pots and each pot was composed of one single plant. A control group of plants was sprayed with distilled water. Tween-20 (0.1%) was added to all solutions as surfactant.

Growth characteristics

One week after the final treatment, the morphological and biochemical parameters were measured.

1. Fresh and dry weight of flowers. To measure fresh weight of flowers, 3 flowers of each pot were detached. Then, their fresh weights were recorded immediately using a digital scale (PJ300, Mettler, France) with 0.0001 g precision. To determine the dry weight of flowers, the samples were first oven-dried at 72°C for 24 h and then, they were re-weighed using a digital scale.

2. Longevity of flowers. To evaluate the longevity of the flowers per plant, the days were counted from anthesis until when the flowers could keep their freshness.

Antioxidant enzymes activity

Kang and Saltveit's method (2002) was used to provide plant extracts to determine the activity of catalase, ascorbate peroxidase and guaiacol peroxidase. The mature leaves on mid stems of plants of each pot (0.5 g fresh weight), were excised rapidly and ground in 3 mL extraction buffer (for 5 min using a pestle and mortar on ice). Buffer solution was included 0.05 mM hydrochloric acid, 3 M magnesium chloride and 1 M EDTA (pH = 7.5). The homogenates were centrifuged at 4,000 rpm for 20 min at 4°C. The supernatant filtered through two layers of cheese-cloth was used for the assays of enzymatic activities.

1. Catalase assay. The activity of CAT was measured using the method of Aebi (1984). The CAT reaction solution including 2.5 mL of 50 mM phosphate buffer (pH = 7) and 0.2 mL H_2O_2 (0.1%) was mixed rapidly with 0.3 mL enzyme extract. Changes in absorption at 240 nm were read after 1 min with a spectrophotometer. The catalase activity was calculated by using the formula:

$$(\text{Units}\frac{mM}{min}) = \frac{doD/min(slope)\times Vol.f\ assay\ (0.0003)}{Extinction\ Coefficient\ (43.6)}$$

2. Ascorbate peroxidase assay. The ascorbate peroxidase (APX) activity was determined according to the method of Nakano and Asada (1981). The reaction solution including (2.5 mL) of 50 mM phosphate buffer (pH = 7), 0.1 mL EDTA, 1 mM sodium ascorbate and 0.2 mL H_2O_2 was mixed rapidly with 0.1 mL enzyme extract. The light absorbance of the reaction solution was read after 1 min at 290 nm. The ascorbate peroxidase activity was calculated by using the formula:

$$(\text{Units}\frac{mM}{min}) = \frac{doD/min(slope)\times Vol.f\ assay\ (0.0003)}{Extinction\ Coefficient\ (2.8)}$$

3. Guaiacol peroxidase assay. The activity of GPX was measured using the method of Updhyaya et al. (1985). The reaction solution including (2.5 mL) of 50 mM phosphate buffer (pH = 7), 1 mL guaiacol (1%), and 1 mL H_2O_2 was mixed rapidly with 0.1 mL enzyme extract. The light absorbance of the reaction solution was read after 1 min at 420 nm. The guaiacol peroxidase activity was calculated by using the formula:

$$(\text{Units}\frac{mM}{min}) = \frac{doD/min(slope)\times Vol.f\ assay\ (0.0003)}{Extinction\ Coefficient\ (26.6)}$$

4. Antioxidant capacity. Antioxidant capacity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical degradation method (Epsin et al., 2000). 50 µL of leaf extract solution was introduced into test tubes, and 1,000 µL of 0.1 mM DPPH solution was added. The tubes were mixed and allowed to stand for 50 min in the dark. Absorbance was read against a blank at 517 nm using a spectrophotometer.

5. Proline content. Proline content was determined according to the method described by Irigoyen et al. (1992). Fresh leaf material (0.5 g) was homogenized in 5 mL of 75% ethanol, and the homogenate was centrifuged at 3,500 rpm. 1 mL of the supernatant was mixed with 5 mL of acid ninhydrin and 5 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 45 min at 100°C. The reaction mixture was extracted with 10 mL benzene, and the homogenate was centrifuged, cooled to room temperature, and the absorbance was measured at 515 nm with a UV/visible spectrophotometer. Appropriate proline standards were included for the calculation of proline in the samples (Paquin and Lechasseur, 1979).

6. Electrolyte leakage and lipid peroxidation assays. Electrolyte leakage (EL) was determined following the method described by Lutts et al. (1995). Ten leaf discs (1 cm) were placed in test tubes containing 24 mL distilled water. The tubes were incubated in a water bath at room temperature for 24 h, and the initial electrical conductivity of the medium (EC1) was analyzed using an electrical conductivity analyzer. The samples were autoclaved at 120°C for 20 min to release all electrolytes, cooled to 25°C, and then the final electrical conductivity (EC2) was measured. The EL was calculated using the formula: EL = (EC1/EC2) × 100.

Lipid peroxidation was estimated as the content of all 2-thiobarbituric acid reactive substances and expressed as equivalents of malondialdehyde (MDA), as described by Cakmak and Horst (1991).

Statistical analysis

The experiment was arranged as a factorial trial based on a completely randomized design. Data were analyzed using analysis of variance (ANOVA) in SAS v. 9.1. Significant differences among the means were declared at $p < 0.05$ or 0.01.

Results

Analysis of variance indicated significant differences by SNP and SA foliar application on all measured parameters but the interaction between them didn't give significant difference (except catalase activity, electrolyte leakage and MDA) (Table 2).

Flowers characteristics

Data presented in Figure 1 indicated that, among different concentrations of SNP, only 1 mM significantly increased flower fresh weight to 1.69 g as compared to the control, and other SNP concentrations did not show any significant differences with untreated plants. Figure 2 shows that foliar application of 1 and 2 mM SA has a potent effect on the fresh weight of flowers of *Rosa hybrida* and makes a significant difference with control.

With respect to the dry weight of flowers of plants, it can also be observed that among all levels of SNP, the concentrations of 1 and 2 mM were related to the highest dry weight,

Table 2. Effects of sodium nitroprusside and salicylic acid on some morphological and biochemical characteristics of *Rosa hybrida* under alkaline stress.

		Mean squares								
Treatments	D.F.	Flower fresh weight	Flower dry weight	Flower longevity	Catalase activity	Ascorbate peroxidase activity	Gayacol peroxidase activity	Antioxidant activity	Proline	Electrolyte leakage	MDA
SA	4	0.311030**	0.019249**	347.308**	9.908508**	24.32272**	15.28371**	320.272**	0.47646^{ns}	176.306**	1.73507**
SNP	3	0.301987*	0.014346**	170.088**	2.633725**	9.26884**	6.68144**	383.723**	1.2331*	187.190**	0.03081**
SA*SNP	12	0.029905^{ns}	0.001707^{ns}	7.797^{ns}	0.348514*	0.6834^{ns}	1.1268^{ns}	35.629^{ns}	0.18309^{ns}	16.502*	0.01859**
Error	40	0.079935	0.00167	23.11	0.1538	0.6779	0.8702	77.586	0.36667	6.99	0.0035
C.V.		18.2	15.2	17.8	11.4	17.9	13.2	12.06	10.6	11.5	9.2

**, *: significant on the level of 1%, *: significant on the level of 5%, ns: not significant.

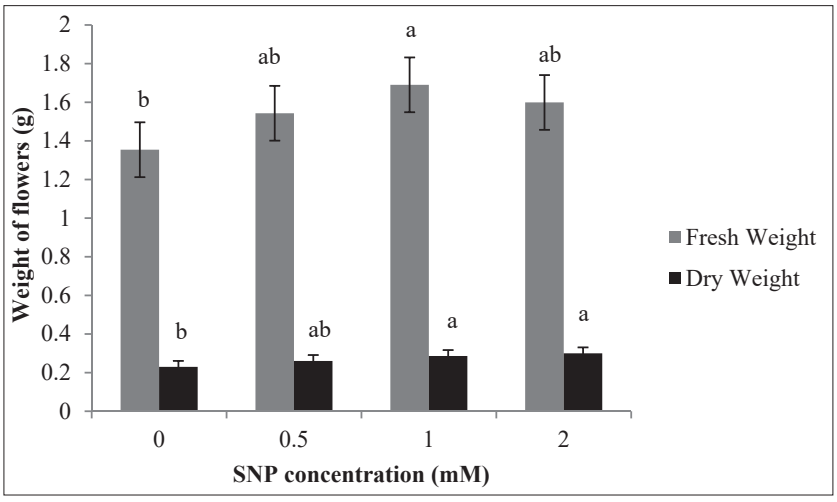

Figure 1. Effect of foliar application of SNP on flowers fresh and dry weight of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 1%).

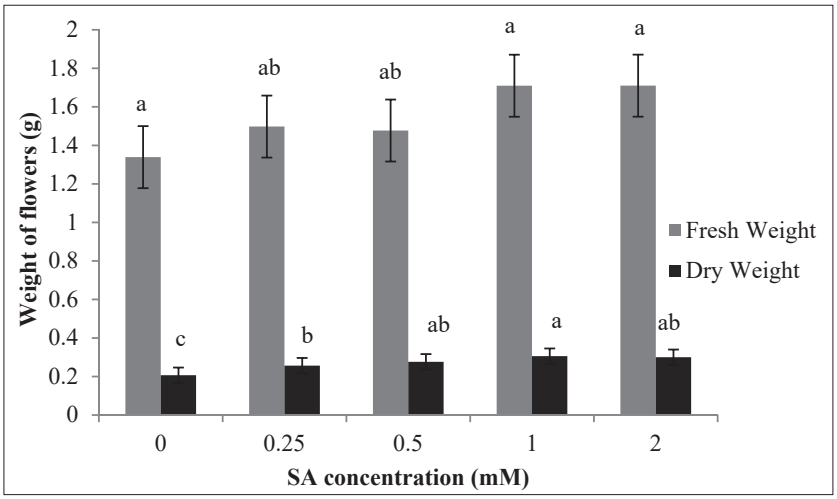

Figure 2. Effect of foliar application of SA on flowers fresh and dry weight of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 1 and 5%).

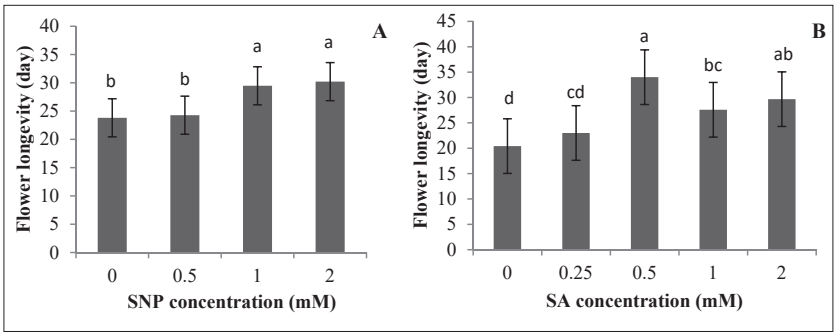

Figure 3. Effect of foliar application of SNP and SA on flower longevity of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 1%).

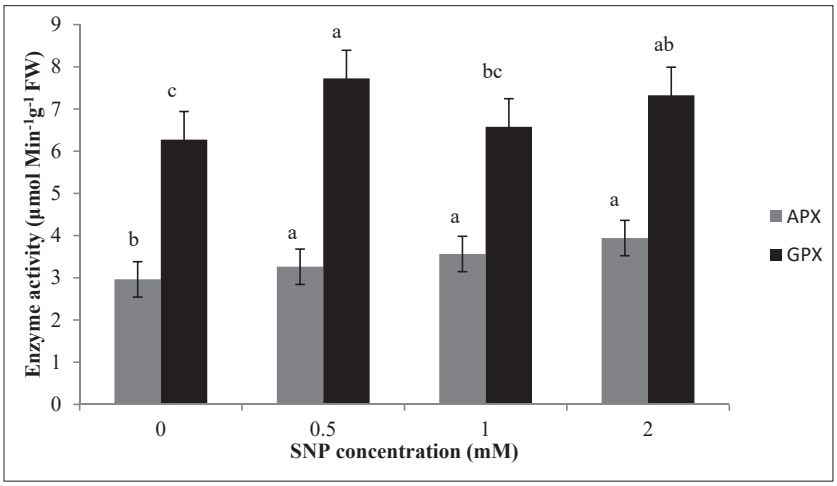

Figure 4. Effect of foliar application of SNP on activity of ascorbate peroxidase and guaiacol peroxidase of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 5%).

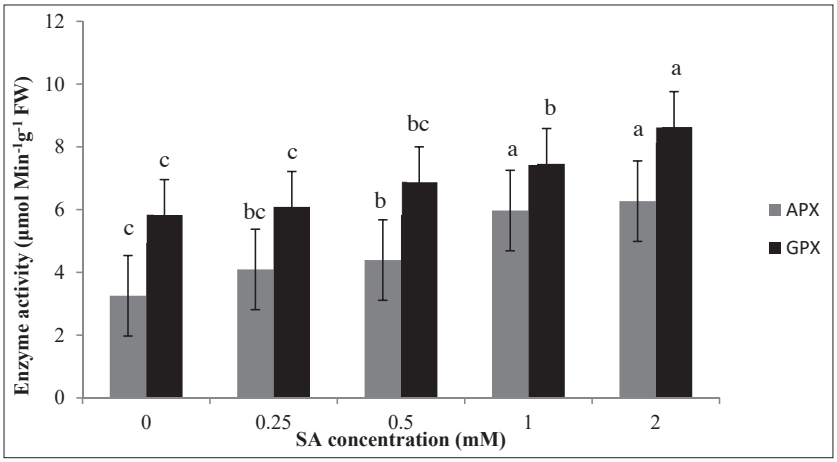

Figure 5. Effect of foliar application of SA on activity of ascorbate peroxidase and guaiacol peroxidase of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 1%).

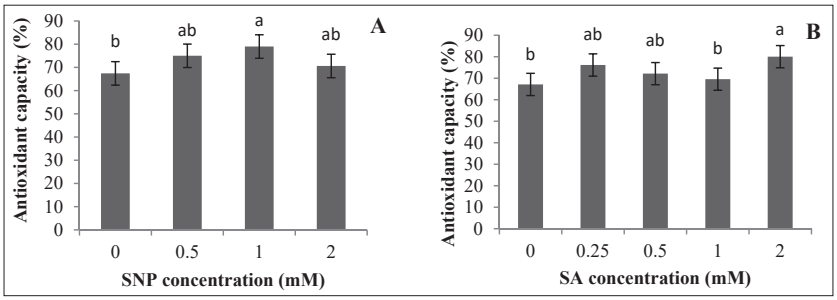

Figure 6. Effect of foliar application of SNP and SA on antioxidant capacity of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 1%).

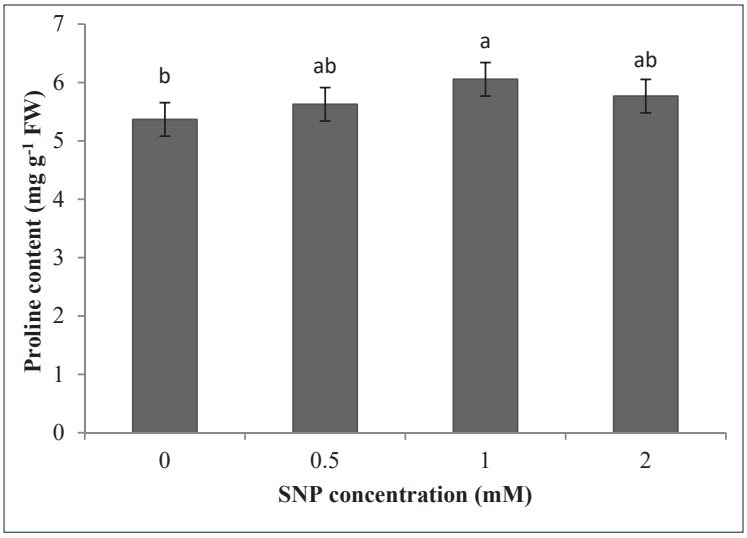

Figure 7. Effect of foliar application of SNP on proline content of *Rosa hybrida* plants grown under alkaline stress. (Common letters showed no significant difference at the level of 5%).

while other concentrations of SNP did not differ significantly with untreated plants (Figure 1). Dry weight of flowers showed an increase significantly in all concentrations of SA (Figure 2).

Analyzing the value of flower longevity under the conditions of present study, the highest increase in the longevity of flowers was observed in the plants treated with 1 and 2 mM SNP (Figure 3A) and 0.5, 1 and 2 mM SA, but there was no significant difference between other treatments and the control plants (Figure 3B).

Antioxidant enzymes activity

Data presented in Table 3 indicates that the spraying of both SNP and SA increased the CAT activity of rose in alkaline conditions. According to Table 3, there is a significant difference between the application of SNP and SA at 2 mM concentrations with the control. The lowest CAT activity was observed in the untreated plants and the highest was obtained from plants treated with 2 mM of SNP and SA.

The results on the effect of different concentrations of SNP and SA on APX activity showed that all concentrations of SNP increased the APX activity of rose plants while all concentrations of SA increased APX activity compared to control except the 0.25 mM (Figure 5).

The results revealed that foliar application of SNP and SA at different concentrations affected GPX activity. The highest increase in GPX activity was observed in response to 0.5 mM SNP (Figure 4) and 2 mM SA treatments (Figure 5). In SNP treatments, no significant difference for enzyme activity was observed between the concentration of 0.5 and 2 mM.

Cell damage assays

The results of means comparison show that foliar application of 1 mM SNP (Figure 6A) and 2 mM SA (Figure 6B) has a potent effect on the antioxidant capacity of *Rosa hybrida* and makes a significant difference with other treatments.

With respect to the proline content of leaves of rose plants, it can also be observed that among all levels of SNP, only the concentration of 1 mM was related to the highest proline content (6.05 µmol g^{-1} F.W.), while other concentrations did not differ significantly from untreated plants (Figure 7).

The results showed that foliar application of SNP and SA led to a significant reduction in electrolyte leakage compared to non-treated ones. The 0.5 mM SA and 2 mM SNP application lowered electrolyte leakage. Results showed that the least EL content (16.92%) was achieved in these treatments (Table 3).

Data presented in Table 3 indicated that the exogenous application of SNP and SA have a positive impact on the adverse effects of stress. Results showed that the least MDA content (0.246 µmol g^{-1} F.W.) was achieved in 0.25 mM SA in all concentrations of SNP.

Discussion

In this research, we analyzed the possible role of exogenous SNP and SA in the modulation of the antioxidant defense system against alkaline stress in rose plants. Evidences have been demonstrated that the interaction between NO and SA had synergistic effects in decreasing of the damages induced by all kinds of abiotic stress (Simaei et al., 2011; Dong et al., 2015). In the present study, we were able to increase the rose antioxidant capacity and its components under alkalinity by applying NO and SA.

Alkalinity stress as like as other stresses has been reported to have negative effects at plant growth which might be due to the formation of harmful reactive oxygen species (ROS) that cause oxidative stress. In this research, foliar application of SNP increased fresh and dry weight of flowers in alkaline soil because of the effects of SNP on scavenging ROS, increasing the activity of antioxidant enzymes, as well

Table 3. The effect of sodium nitroprusside (SNP) and salicylic acid (SA) on catalase activity, electrolyte leakage and MDA content of rose 'Beverly Watson'.

SNP	SA	Catalase activity	Electrolyte leakage	MDA content
0	0	1.47 ± 0.07460684^{j}	34.99 ± 0.10000000^{a}	1.54333 ± 0.02253886^{a}
0	0.25	2.4297 ± 0.58959023^{b-e}	28.943 ± 1.19500349^{ac}	0.29767 ± 0.00300000^{e}
0	0.5	3.0917 ± 0.46525388^{hi}	24.44 ± 2.99601402^{bcd}	0.47767 ± 0.00351188^{d}
0	1	3.9804 ± 0.26759749^{ei}	23.63 ± 0.67500617^{cde}	0.65433 ± 0.00351188^{c}
0	2	3.8396 ± 0.37536483^{b-f}	23.78 ± 3.52000000^{cde}	0.581 ± 0.00300000^{cd}
0.5	0	2.1336 ± 1.03230346^{ij}	33.09 ± 0.20000000^{a}	1.17 ± 0.01000000^{b}
0.5	0.25	2.8693 ± 0.32495278^{bcd}	22.98 ± 2.44500170^{cde}	0.29767 ± 0.00700000^{e}
0.5	0.5	3.2216 ± 0.91315500^{f-i}	21.017 ± 1.44576393^{de}	0.46067 ± 0.00351188^{d}
0.5	1	4.1234 ± 0.15725702^{d-h}	20.737 ± 3.11000000^{de}	0.67733 ± 0.00351188^{c}
0.5	2	3.9576 ± 1.01684784^{b-e}	20.017 ± 0.90500460^{de}	0.56 ± 0.00888819^{cd}
1	0	2.8852 ± 1.15319743^{f-i}	30.273 ± 3.03500137^{ab}	1.15 ± 0.01000000^{b}
1	0.25	2.9384 ± 0.15915234^{bc}	21.263 ± 2.16584856^{de}	0.24667 ± 0.00700000^{e}
1	0.5	3.4327 ± 0.10378913^{f-i}	21.03 ± 1.17099673^{de}	0.45233 ± 0.02253886^{d}
1	1	4.2118 ± 0.04864895^{c-g}	21.853 ± 2.88098941^{de}	0.64567 ± 0.02253886^{c}
1	2	4.3521 ± 0.62105704^{bc}	18.683 ± 0.90500460^{de}	0.56 ± 0.02253886^{cd}
2	0	2.818 ± 0.14596098^{ghi}	19.81 ± 3.78000000^{de}	1.23 ± 0.01527525^{b}
2	0.25	3.0529 ± 0.41842337^{b}	18.713 ± 2.52010582^{de}	0.24233 ± 0.00611010^{e}
2	0.5	3.5284 ± 1.44989417^{e-i}	16.92 ± 0.50000000^{e}	0.439 ± 0.00416333^{d}
2	1	4.6317 ± 0.85106672^{c-g}	20.067 ± 3.18961335^{de}	0.66867 ± 0.01331666^{c}
2	2	5.676 ± 1.28464664^{a}	17.3 ± 0.85440037^{e}	0.55933 ± 0.01890326^{cd}

In each column, values followed by the same letter(s) do not differ significantly at $\alpha = 0.01$ and 0.05.

as levels of proline which led to improved plant growth. These results are similar to previous studies (Ghadakchiasl et al., 2017; Zhang et al., 2007). Also, we found that fresh and dry weight of flowers was increased by SA treatment as compared to control. In relation to reducing the effects of alkaline stress by SA, Rajeshwari and Bhuvaneshwari (2017) exhibited that SA as a signaling molecule can help plants to cope with abiotic stresses. Improved plant growth, ion absorption and transfer, prevent of oxidative damage in the plant by detoxifying superoxide radicals have been reported by application of SA on different plant species (Shahmoradi and Naderi, 2018). Our results are supported by those of Javaheri et al. (2012) which founded that foliar application of SA enhanced the flowering and leaf area of tomato plants. Also, Li et al. (2014) reported that salicylic acid induced stress tolerance and increased biomass of *Torreya grandis* as a result of enhanced chlorophyll content and the activity of antioxidant enzymes that eventually activated the photosynthetic process and alleviated oxidative stress. In literature it was reported that both application of SNP and SA significantly increase the absorption of water and nutrients with increasing root volume, leaf area and photosynthetic pigments (Bayat et al., 2012; Moazam Babasheikhali et al., 2020). When the amount of dry matter produced in the plant was increased, therefore, these cases may affect surface and thickness of the petals, and this will increase the fresh and dry weight of the flowers. In accordance with these results, the use of SNP and SA in increasing the resistance of *Pinus eldarica* to salinity and improving its growth characteristics was reported by Zamani et al. (2014).

One of the compounds that are synthesized in plants in abiotic stress is ethylene (Khan et al., 2017). Nitric oxide delays senescence of flowers by reducing ethylene production due to inhibition of ACC synthase activity and decreased ACC content. It has also been shown that nitric oxide, as an antioxidant, delays the senescence of plant tissues. The results have shown that salicylic acid, by increasing the activity of antioxidant enzymes, adjusts amount of H_2O_2 in the plant and also causes stomata closure then, thus reducing transpiration rates and preserving water and delaying senescence in flowers (Alaey et al., 2011; Hayat et al., 2010a).

The results of this study showed that the application of SNP increased the activity of GPX, CAT and APX in rose, thereby ameliorating the ability to scavenge free radicals and reducing the injury. Similar results have been seen in *Solanum lycopersicum* at sodic alkaline stress (Gong et al., 2014). It is highly possible that the protective effect of NO may be mediated by increased level of expression of genes encoding active oxygen scavenging enzymes under stress (Shi et al., 2005). In addition, NO could modify the activity of protective enzymes. The main reason was that NO had a strong desire for the enzymes containing iron that improve the ability of the protective enzyme activity. NO also, through direct interaction with ROS, reduced its production, and then producing peroxy nitrite radical ($ONOO^-$), which is an unstable product and less toxic, thus causes less damage than the original superoxide anions (Fan et al., 2014). As mentioned above, SNP is a releasing compound of NO in plants, and acts as a signaling molecule to increase the activity of antioxidant enzymes such as SOD and CAT (Saed-Moucheshi et al., 2014); this protects proteins and lipids against free radicals (Ghadakchiasl et al., 2017). Nitric oxide has a dual function as a potent oxidant and effective antioxidant (Hayat et al., 2010b). The antioxidant role of NO is mainly supported by its ability to protect the cellular redox homeostasis and adjust the toxicity of ROS (Sheokand and Kumari, 2015).

In the present study, foliar application of SA increased the activity of the antioxidant enzymes in rose plants. Exogenous SA can increase H_2O_2 content of tissues, induce the expression of antioxidant enzymes, and increase plant tolerance to the abiotic stressors (Szepesi et al., 2008). Both endogenous and exogenous SA was evidenced to play roles in antioxidant metabolism and have a tight control over cellular ROS (Kong et al., 2014; Khan et al., 2014). However, the coordination of SA-dependent and independent signaling components with ROS-signaling provided an appropriate defense response (Iqbal et al., 2015).

In this research, exogenous SNP increased proline content under alkalinity stress. In agreement with the present study, application of SNP has been shown to increase the levels of compatible solutes such as proline and glutathione. In agreement with the present study, application of SNP has been shown to increase the levels of compatible solutes such as proline and glutathione (Tan et al., 2008). Lei et al. (2007) demonstrated that NO induced and increased the activity of pyrolin-5-carboxylate synthetase (P5Cs) in the synthesis of proline in wheat under drought stress.

In this study, foliar application of SNP and SA reduced the electrolyte leakage. Alkaline stress damaged the cellular membranes. This damage caused increased electrolyte leakage and increased level of lipid peroxidation. Alkaline stress induces lipid peroxidation through reactive oxygen species production. Thus exogenously applied SNP (NO donor) and SA had a protective effect on alkaline-induced membrane damage. Studies suggested that NO effectively reduces the level of ROS generated during stress, and thus, limits oxidative damage in plant cells (Arasimowicz and Floryszak-Wieczorek, 2007), while SA facilitated maintenance of membrane functions through induction of antioxidant mechanisms and elevated ion uptake, thereby protecting the plants against the oxidative damage (El-Tayeb, 2005). According to the cited authors, the application of NO and SA, which facilitated maintenance of membrane functions under abiotic stress, could be an indicator of the build-up of a protective mechanism to reduce cytoplasm fluid leakage induced by abiotic stress (Dong et al., 2015).

Application of NO and/or SA reduced alkaline-induced increase the content of MDA (Table 3). It was suggested that superoxide anion ($O_2^{\bullet-}$), H_2O_2, radicals of hydroxyl ($OH^{\bullet}$), lipid alcoxyl ($LO^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$), which are produced during stress, can be the major factors responsible for lipid peroxidation. The level of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Li et al., 2008). It has been mentioned that the reaction of NO with lipid alkoxyl (LO) and peroxyl (LOO) radicals is fast, and could thus prevent the propagation of radical-mediated lipid oxidation in a linear fashion (Sheokand et al., 2008). SA was reported to inhibit production of hydroxyl radical and to decrease the content of MDA (Gunes et al., 2005). Proline is able to scavenge hydroxyl radical and stabilize the membrane structure (Misra and Saxena, 2009). In literature it is cited that SA, through the induction of ABA-mediated proline production, can decrease the deleterious effects of salt stress and water deficit in wheat seedlings (Sakhabutdinova et al., 2003). Because MDA and proline are often considered a compatible solute involved in osmotic adjustment under stress, reduction in the level of MDA and increase in the level of proline in (NO+SA)-treated plants indicate the stress amelioration role of NO and SA that might be responsible for maintenance of plant growth under stress (Dong et al., 2015).

Conclusion

In conclusion, SNP combined with SA could effectively alleviate the injury induced by alkaline during the developmental stages of the rose plants. Likely, one of the mechanisms for alkaline tolerance improvement by NO and SA is associated with their high capacity to decrease free radical production and membrane damage by maintaining the high antioxidant enzyme activities. Finally, in most of the indices measured, high concentrations of NO and SA treatments including (1 and 2 mM) showed the best effect, and it was observed that they had synergistic effects in CAT activity, electrolyte leakage, and MDA content.

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