The effect of different concentrations of exogenous nitric oxide on several physiological and biochemical parameters in NaCl-stressed coriander (*Coriandrum sativum* L.)

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Abstract

In this study the influence of sodium nitroprusside (SNP, the donor of NO) was investigated on several physiological and biochemical parameters in *Coriandrum sativum* L. grown in saline and non-saline conditions. Fifteen-day-old coriander seedlings were treated with 50 and 100 mM NaCl and 50, 75, and 100 µM sodium nitroprusside during 3 months. Then, carotenoids, carbohydrate, and soluble protein contents and proline accumulation were measured. Results indicated that NaCl-induced ionic toxicity led to a decrease in carotenoids amount. Under NaCl salinity, carbohydrate content increased sharply as compared with control plants. The protein content of plants did not follow a determined pattern. Furthermore, results showed that NaCl-induced ionic toxicity led to a significant increase in proline accumulation. Application of 50 µM SNP could improve carotenoids content of coriander. The use of different concentrations of SNP showed various effects on carbohydrate content. Application of 50 µM of SNP significantly enhanced the total protein content and proline accumulation; application of 75 and 100 µM SNP had variable effects on all measured parameters. These results suggested that 50 µM of SNP is suitable for reducing damage associated with salt stress.

Keywords: NaCl salinity; nitric oxide; biochemical parameters; physiological parameters; *Coriandrum sativum* L.

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Introduction

Coriander (*Coriandrum sativum* L.), a culinary and medicinal plant of Apiaceae (Umbelliferae) family, is an annual and herbaceous plant originated from the Mediterranean and Middle Eastern regions (Veena and Manu, 2014). All parts of this herbal medicine were used as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations around the world (Sahib et al., 2013). Plants are usually exposed to different environmental stresses

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during their development which cause many common reactions such as limiting their growth and productivity and having a substantial impact on agricultural production worldwide (Shao et al., 2008). One of the most important environmental stresses is salt stress, which imposes ion toxicity and osmotic stress on plants, leading to nutrition disorder and oxidative stress. In many cases, the primary source of salt stress-induced oxidative bursts is the uncontrolled ROS accumulation, provoking damage to macromolecules, or even to the antioxidative system (Manaa et al., 2013). Plants have evolved a specific antioxidant protective system consisting of enzymes such as superoxide dismutase (SOD), catalase (CAT), and non-enzymatic constituents such as ascorbate and glutathione, which are responsible for scavenging excessively accumulated ROS in plants under stress conditions (Jung et al., 2000). Accordingly, the regulation of the antioxidant constituents through exogenous substances might enhance the plant tolerance to salt stress (Shi et al. 2007).

Recent plant molecular studies have shown that Nitric oxide (NO), as a versatile molecule, is involved in a wide spectrum of physiological processes from bacteria to human. NO has an important role in growth and development of higher plants, including seed germination, senescence, defense response, and abiotic stresses. It is also a key signaling molecule in different intracellular processes (Corpas et al., 2011). In relation to abiotic stresses, it was shown that the application of SNP (sodium nitroprusside), an NO donor, abates the harmful effects of salinity (Lopez-Carrion et al., 2008). Furthermore, as an antioxidant agent, NO can scavenge ROS as a signaling molecule leading to alterations of antioxidative gene expression, and thus function to protect plant cells from oxidative damage (Arasimowicz and Floryszak-Wieczorek, 2007). However, studies have shown that nitric oxide is a gaseous reactive nitrogen species, and its effect on different cells is either protective or toxic, based on its concentration and position of action (Lamattina et al., 2003).

In this work, we investigated the effect of different concentrations of nitric oxide on several physiological and biochemical parameters of coriander in both salt and not salt stress conditions for determination of the best concentration of NO for alleviation of salt stress.

Materials and Methods

Plant growth and treatment

Seeds of coriander (*Coriandrum sativum* L.) were surface sterilized by dipping in 0.5% (v/v) sodium hypochlorite for 5 min., and then washed thoroughly with sterile distilled water for several times. Sterilized coriander seeds were placed between two layers of filter paper in petri dishes containing distilled water before they were kept at 25° C under dark in an incubator. The water in petri dishes were regulated every day. After germination, 15-day-old seedlings were planted in a hydroponic system in the greenhouse (Hoagland solution (Hoagland and Arnon, 1957), a 14-h light, day/night temperatures of 25° C/20° C, and 50-60% relative humidity). Flower pots were randomly arranged in a greenhouse during the treatment period. Sodium chloride (0, 50, and 100 mM) with or without SNP (0, 50, 75, and 100 µM) was added to Hoagland solution during the growth. SNP (Merck, Germany) was used as a donor of NO. The Hoagland solution was renewed 3 times each week, and the pH was adjusted near 6.5 (Hoagland and Arnon, 1957). At the end of the growth stage, the plants were harvested, and roots and shoots were separated and washed with deionized distilled water. To determine some parameters, fresh plant material immediately were frozen by liquid nitrogen and then stored at -20° C. For each treatment, three replicates were considered.

Determination of carotenoids content

The content of carotenoids (xanthophylls and carotenes) were determined according to the procedure described by Lichtenthaler and Wellburn (1983). The photosynthetic pigments were extracted from 0.1 g leaf fresh weight by 80% acetone. Then the homogeneous mass was filtered through Whatman No. 1 filter paper. Using a spectrophotometer, absorbance at 2.663 nm (chlorophyll a), 8.646 nm (Chlorophyll b) and 470 nm (carotenoids) was measured and 80% acetone was used as the control to set zero absorbance spectrophotometer.

For assay of carotenoids, we used the following equation:

Carotenoids (x+c)[1]= (1000 A_{470}[2] -1.8 Chl_a[3] – 85.02 Chl_b[4])/198

Where (x+c): xanthophylls and carotenes, A_{470}: absorbance at 470 nm, Chl_a: chlorophyll a content, and Chl_b: chlorophyll b content.

Determination of carbohydrate amount

Determination of carbohydrate contents was carried out by Cu^{+2} reduction test according to Nelson (1944) and Somogyi (1952) method. Two mL of the extract (0.05 g ground fresh leaf, 5 mL water) were mixed with 2 mL copper reagent of Somogyi. The mixtures were transferred to a boiling water bath for 20 minutes. After cooling, 2 mL Nelson's arsenomolybdatere agent was added. After 10 minutes, the absorbance of the reaction mixture was measured at 600 nm using a spectrophotometer. The amounts of carbohydrates were calculated by a standard curve obtained from different concentrations of standard glucose solution.

Protein extraction and assay

Frozen leaf samples (0.5 g) were used for protein extraction according to the method introduced by Bradford (1976). Samples were ground and lixiviated to 5 ml of 50 mM phosphate buffer (pH 7.5), using a pre-chilled mortar with a pestle. The phosphate buffer contained 1 mM EDTA, 1 mM PMSF, and 1% PVP-40. This was followed by centrifugation at 4° C at 15,000 × g for 30 min. The supernatant was used to measure protein concentration.

To assay total protein content, 1 ml of the supernatant was added to 5 ml Coomassie Brilliant Blue G-250 reagent. After 20 min, the solution absorbance was read at 595 nm, and the activity was calculated using the standard bovine serum albumin (BSA) (Bradford, 1976).

Proline content determination

Proline content determination proceeded according to Bates et al. (1973). Leaf tissue (5 g) was homogenized in 3% sulfosalicylic acid, and then filtered. To 2 ml of the filtrated solution, 2 ml of ninhydrin acid and 2 ml of glacial acetic acid were added, and the mixture was incubated for 1 h in a boiling water bath, which was followed by an ice bath. To do this, 4 ml toluene was added and mixed vigorously. Afterward, the chromophore containing toluene was separated from the aqueous phase and the absorbance was measured at 520 nm. A standard curve was established using known concentrations of authentic proline, and was calculated as µM g^{-1} FW.

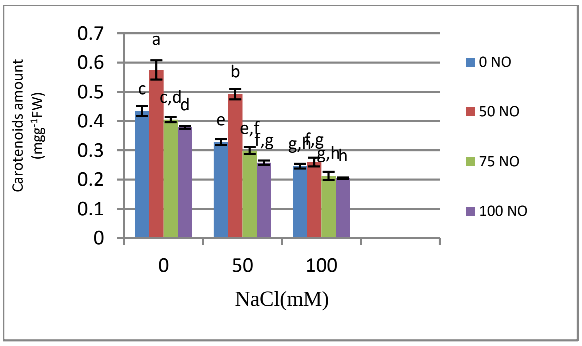

Fig. I. Effects of NO (µM) on the carotenoids content in coriander under saline and non-saline conditions; results are shown as mean ± SE ($p<0.05$), obtained from three replicates. Means that do not share a letter are significantly different.

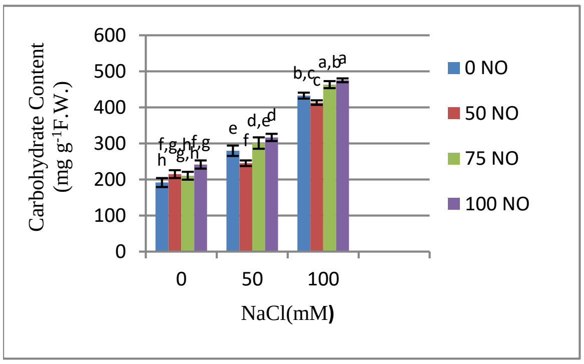

Fig. II. Effects of NO (µM) on the carbohydrate content in coriander under saline and non-saline conditions; results are shown as mean ± SE ($p<0.05$), obtained from three replicates. Means that do not share a letter are significantly different.

Statistical analysis

Data were analyzed using ANOVA (completely randomized) to determine if there were significant differences among the obtained means. Duncan's multiple range tests were carried out to determine if there were significant differences ($P \leq 0.05$) between individual treatments (SPSS-13).

Results

Carotenoid contents

As shown in Fig. (I) when NaCl concentration increases, the content of carotenoids significantly reduced. Under salinity and non-salinity conditions, the amount of carotenoids increased by 50 µM SNP. In this condition, 75 and 100 µM SNP, decreased content of carotenoids.

Carbohydrate content

As shown in Fig. (II), a significant increase in carbohydrate content was observed in response to 50 and 100 mMNaCl salinity as compared with control plants. Our data showed that application of 50 µM SNP enhanced the carbohydrate content in non-saline condition, but, it reduced carbohydrate content in saline condition. In all concentrations of NaCl, application of 75 and 100 µM SNP, enhanced the carbohydrate content of plants.

Protein content

Results indicated that the amount of protein does not follow a specific pattern (Fig. III). Thus, the addition of 50 mM NaCl to the medium increased significantly the amount of total protein while the addition of 100 mM NaCl decreased it. In the absence of salinity medium, the application of all three concentrations of nitric oxide (50, 75, and 100 µM) significantly increased the amount of protein. In the presence of 50 mM NaCl, addition of 50 µM NO significantly increased protein amount while the addition of 75 and 100 µM NO significantly decreased it. In the medium containing 100 mM NaCl, application of 50 µM NO significantly increased the total protein content; however, both 75 and 100 µM nitric oxide significantly decreased the amount of protein.

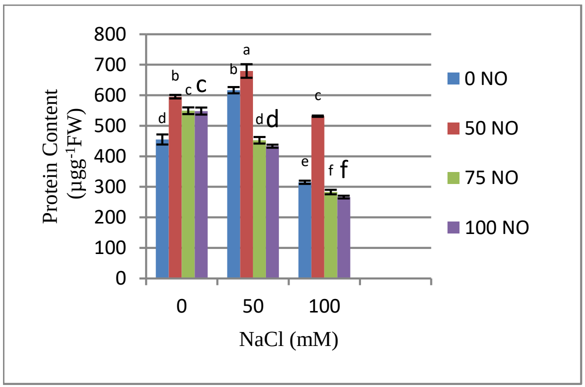

Fig. III. Effects of NO(µM) on the protein amount in coriander under salin and non-salin conditions. Results are shown as Mean ± SE ($p<0.05$), obtained from three replicates. Means that do not share a letter are significantly different.

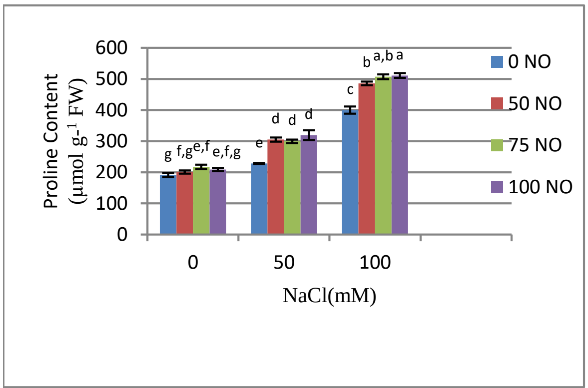

Fig. IV. Effects of NO (µM) on proline content in coriander under salin and non-salin conditions. Results are shown as Mean ± SE ($p<0.05$), obtained from three replicates. Means that do not share a letter are significantly different.

Proline concentration

Results showed that proline concentrations at 50 mM and 100 mM NaCl were enhanced up to 19.37% and 109.42%, respectively, compared to control plants. As shown in Fig. (IV), a significant increase in free proline accumulation was observed in response to 50 and 100 mM NaCl salinity. Our data showed that the amount of proline was enhanced by NO under saline and non-saline conditions. The effect of nitric oxide on the increasing proline content was more pronounced in the saline medium than the non-saline medium.

Discussion

Although there is a plethora of plant reactions to circumvent the harmful effects of environmental conditions, a wide range of stresses, such as high and low temperature, drought, alkalinity, salinity, UV stress, and pathogen infection are potentially harmful to the plants (Van Breusegem et al., 2001). Salinity of soil or water is one of the major stress obstacles, especially in arid and semi-arid regions, which can severely limit plant growth and productivity. Coriander as a culinary and medicinal plant is one of the most consumed vegetables in the world. However, it is extremely sensitive to different adverse environmental conditions, including salinity, which provokes a significant reduction in crop productivity (Neffati et al., 2011).

Plants have developed a wide variety of defense strategies to combat oxidative damage. There are many lines of evidence indicating that NO plays important roles in plant tolerance to environmental stress, including salt stress (Uchida et al., 2002), drought stress (Arasimowicz-Jelonek et al., 2009), heavy metal toxicity (Xu et al., 2010), etc. Research findings suggest that NO alleviates abiotic stress through different metabolism, and antioxidant capacity modulation is reported to be one of the most important pathways in many investigations (Hao et al., 2009).

Our results showed that salinity reduced the carotenoids content in leaves of coriander plants. Furthermore, the effect of severe salinity is higher than low salinity. It has been observed that exogenous application of 50 µM SNP increases the content of photosynthetic pigments under 50 mM NaCl salinity, but it had no positive effect under 100 mM NaCl salinity. According to our findings, high concentration of SNP has a positive effect and mitigates the damaging effects of salt. It has been suggested that exogenous NO leads to the promotion of protective reactions to the photosynthetic pigments and induces a pre-adaptive 25response to salt stress (El-Tayeb, 2005), which confirm our results.

The most important molecule which affects different physiological responses is sugar (Crowe et al. 1990). Accumulation of carbohydrate in plants' tissue under condition of environmental stress is a result of regulation and modification in current stress (Dhanapackiam, and Ilyas, 2010). Generally, the increase in cellular osmolality which is achieved from accumulation of compatible solutes is associated with the influx of water into cell (Hare et al. 1998). So, increasing sugar under environmental stress could be the result of starch decay, sugar synthesis by non-photosynthesis pathways, non-converting of sugar to other productions, and decrease in transportation from leaves to other parts of plant (Premachander et al. 1991).

Proteins play important roles in salt stress acclimation and plant cellular adjustment since proteins perform a vast array of functions within living organisms, including signaling, regulation of gene expression and protein metabolism, defense-related proteins, mechanical stress-related proteins, and secondary metabolism (Kosova et al., 2013). In this study, total soluble protein concentration increased in plants under 50 mM NaCl concentrations. These effects may be due to the synthesis of proteins required to protect the plant against salt stress. However, in response to increasing salinity, harmful effects were increased, many metabolic pathways were inhibited, and most proteins were degraded.

Finally, results showed the reduction of total protein contents under high stress. A decrease in protein concentration was observed in plants grown under high concentrations of NaCl (100 mM). Our results is similar to those of Agastian et al. (2000) who reported that soluble protein increases at low salinity, and decreases at high salinity in *mulberry* cultivars. Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen which is re-utilized later (Singh et al., 1987), and may play a role in osmotic adjustment. They may show de novo synthesis in response to salt stress or may be present constitutively at low concentrations, and increase when exposed to salinity stress (Pareek et al., 1997).

In the present study, exogenous 50 µM NO was able to significantly increase the protein content at all salt concentrations, but 75 µM and 100 µM NO had a reverse effect under salt stress condition. Zhang et al. (2010) showed that the inhibition of NO accumulation decreased soluble protein content in salt-stressed wild plants, which is in congruence with our findings. At lower amounts, NO acts as signals for the activation of defense responses; however, higher concentrations of NO produced from uncontrolled ROS generation, resulted in severe damages.

Proline accumulates under salt stress act as an adjustment osmolyte in plants. It can scavenge ROS, elevate anti oxidation ability, stabilize the structure of macromolecule, decrease the cellular acidification, and detoxify ammonia toxic (Ruan et al., 2002). Our results showed that proline content significantly increased in plants exposed to NaCl stress. The results also indicated that the amount of proline was enhanced by NO under saline and non-saline conditions. The effect of nitric oxide on increasing the amount of proline was highly pronounced in the saline medium compared with non-saline medium. According to Zong et al. (2001), the role of proline in alleviating the negative effects of drought and salt stress in rice might be related to Ca^{2+}, and that NO signaling transduction included Ca^{2+} signaling. They concluded that the protective effects of NO on salt stress-induced oxidative damage in wheat leaves were related to the regulation roles in proline levels.

To sum up, the present study provided evidence that exogenous 50 µM NO alleviated NaCl-induced oxidative stress in *Coriandrum sativum*. Moreover, results indicated that exogenous75 and 100 µM NO had no positive effect on coriander under salt stress condition.

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