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Mitigation of nickel stress by the exogenous application of salicylic acid and nitric oxide in wheat

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Abstract

Nitric oxide and salicylic acid are signaling and ubiquitous bioactive molecules that regulate cellular mechanism in plants under abiotic stress. Therefore, the present experiment was conducted to study the interactive effect of NO and/or SA in tolerance of wheat (*Triticum aestivum* L. cv. Samma) to nickel stress. The treatments were given as follows: (1) Ni_0 mM + NO_0 mM+ SA_0 mM (control), (2) Ni_1 mM + NO_0 mM+ SA_0 mM, (3) Ni_0 mM + $NO_{0.5}$ mM+ SA_0 mM, (4) Ni_0 mM + NO_0 mM+ $SA_{0.5}$mM, (5) Ni_1 mM + $NO_{0.5}$ mM+ SA_0 mM, (6) Ni_1 mM + NO_0 mM+ $SA_{0.5}$ mM, (7) Ni_0 mM + $NO_{0.5}$ mM+ $SA_{0.5}$ mM, (8) Ni_1 mM + $NO_{0.5}$ mM+ $SA_{0.5}$ mM. The results showed Ni-fed plants exhibited moderate reduction in growth characteristics (PH, FW, DW and LA). Also, the application of Ni inhibited CA activity, content of essential elements (N, P, K) and Chl. Plants showed higher accumulation of Ni and MDA under Ni stress. Under Ni stress, the combined application of NO and SA induced the activities of enzymes (CA, SOD, POD, CAT) and accumulation of osmoprotectant proline (Pro). Also, combined application of SA and NO improved the photosynthetic pigments (Chl *a* and Chl *b*) and maintained nutrients homeostasis in plants under Ni-stress. Application of NO and SA together was more effective in suppression of deleterious effect of Ni stress by reducing MDA formation in plants. The present study reveals that application of NO and SA together improved plant growth and development by improving activity of antioxidant enzymes, CA and balance supply of nutrients.

Keywords: nickel stress, photosynthetic pigments, *Triticum aestivum* L., essential nutrients, carbonic anhydrase, antioxidant enzyme.
Abbreviation: APX-Ascorbate peroxidase, CA-Carboninc anhydrase, CAT-Catalase, Chl-Chlorophyll, DDW-Double distilled water, DW-Dry weight, FW-Fresh weight, GB-glycine betaine, GR-Glutathione reductase, LA-Leaf area, MDA-Malondialdehide, Ni-Nickel, NO-Nitric oxide, PH-Plant height, POD-Peroxidase, Pro-Proline, ROS-Reactive oxygen species, RSNOs-S-nitrosothiols, SA-Salicylic acid, SNP-Sodium-nitroprusside, SOD-Superoxide dismutase.

Introduction

Heavy metal pollution is a serious worldwide problem because of their bad impact on human health and environment. Among the heavy metals, nickel (Ni) stress limits the crop productivity. It is considered as a great threat to the world agriculture. Asia, Europe, and North America are suffering from Ni contamination (Zarcinas et al., 2004a, b; Chen et al., 2009; Viet et al., 2010). Nickel is released into the environment from anthropogenic activities, such as metal mining, smelting, fossil fuel burning, vehicle emissions, disposal of house-hold, municipal, industrial wastes, fertilizer application and organic manures (Greeger, 1999). In comparison to other toxic heavy metals such as copper, cadmium, lead, mercury and chromium, Ni has received little attention from researchers because of its dual nature and complex electronic chemistry which is a main barrier in unveiling its toxicity mechanism in plants (Yusuf et al., 2011). Nickel is considered as an essential micronutrients required (in small quantity 0.05–5 μg g^{-1} dry weight) by plants (Seregin and Kozhevnikova, 2006). Presence of Ni at low levels in soil improves plant growth and development by activating several enzymes (Welch, 1981; Andreeva et al., 2001). However, it causes toxicity to plants when it is present in high concentrations in soil (Seregin and Kozhevnikova, 2006). It has been cleared from several studies that high concentrations of Ni cause many toxic effects on plants such as inhibition of seed germination and seedling growth (Ahmad et al., 2009), impairment of growth characteristics (plant height, root length, fresh and dry weight) (Siddiqui et al., 2011b), reduction of root growth due to inhibition of mitotic activity (Gajewska et al., 2006), suppression of photosynthesis due to the disrupted chloroplast structure, blocked chlorophyll synthesis, disordered electron transport, and CO_2 deficit caused by stomatal closure (Ewais, 1997; Sheoran et al., 1990) and inhibition of carboninc anhydrase (CA) activity (Siddiqui et al., 2011b). Also, high level of Ni in plant induces production of highly toxic oxygen species that cause membrane destabilization (Siddiqui et al., 2011a) and disturbs various physiological functions in plants by affecting several enzymes (Welch, 1981; Seregin and Kozhevnikova, 2006). Therefore, to sustain physiological and biochemical processes under stress conditions, plants develop an enzymatic protective mechanism that comprises of antioxidant enzymes peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) that help in the detoxification of reactive oxygen species (ROS). To fight against abiotic stress, plants also develops defence mechanisms. Osmotic adjustment is one of the key mechanisms through which plant

can stand under different environmental conditions by uptaking inorganic ions as well as the accumulation of compatible solutes (osmoprotectants) such as proline and glycine betaine (GB) (Siddiqui et al., 2010, Siddiqui et al., 2011a, 2012a). Under stress, proline in plant behaves like an osmolyte and antioxidant, as well as a source of energy, reducing equivalent, N and carbon (Kuznetsov and Shevyakova, 1997; Matysik et al., 2002). Nitric oxide (NO) is an important signalling molecule with diverse physiological functions in plants. It has been found to play a crucial role in plant growth and development, starting from germination to flowering, ripening of fruit, senescence of organs and defence response to abiotic and biotic stress (Shapiro, 2005; Siddiqui et al., 2011a; Khan et al., 2012). It is a signaling molecule, acts mainly against oxidative stress and also plays a role in plant pathogen interactions. Several lines of study have shown that the protective effect of NO against abiotic stress is closely related to the NO mediated reduction of ROS in plants (Hsu and Kao, 2004). An important biological reaction of nitric oxide is S-nitrosylation, the conversion of thiol groups, including cysteine residues in proteins, to form S-nitrosothiols. S-nitrosylation is a mechanism for dynamic, post-translational regulation of most or all major classes of protein. Nitric oxide has been receiving much attention by researchers, mainly due to its properties (free radical, small size, no charge, short-lived, and highly diffusible across biological membranes) and multifunctional roles in plant growth, development, and regulation of remarkable spectrum of plant cellular mechanisms. However, its function against the stress is still contradictory and unclear, and need to be done (Qiao and Fan, 2008; Siddiqui et al., 2011a). The role of salicylic acid in the mitigation of biotic stress has already been well documented (Horváth et al., 2007). However, SA, as an essential component, is considered as a true plant growth regulator that plays a key role in the signal transduction pathway of abiotic stress (Horváth et al., 2007; Yuan and Lin, 2008). Several earlier findings indicated that SA is involved in the induction of defense mechanisms by modulating the plant response to different environmental conditions. Exogenous application of SA improves the tolerance of plants to salt stress (Yuan and Lin, 2008; Al-Whaibi et al., 2012), to heavy metals (Mishra and Choudhuri, 1999; Pál et al., 2002), to water stress (Singh and Usha, 2003) and to temperature stress (Wang and Li, 2006). According to Yuan and Lin (2008) the involvement of SA in tolerance of plant to abiotic stress has been found contradictory, and the actual defense mechanism of SA in different environment stress remains unresolved. Therefore, it is interesting to explore interactive role of SA and NO in the induction of protective mechanisms against Ni stress. In our knowledge no other studies are found on the combined effect of SA and NO on monocotyledon plants under Ni^{2+} stress. In view of the available evidence, the aim of the present experiment was designed to study the involvement of NO and/or SA in alleviation of Ni^{2+} stress by modulating growth, physiological and biochemical characteristics of wheat.

Results

Plant morphological characteristics

Performance of *T. aestivum* L. was affected adversely compared to the control when plants subjected to Ni, but application of NO and SA alone as well as in combination enhanced growth attributes of plants (Fig. 1A, B and C). The data given in Figure 1 (A-C) show that plants grown in medium containing Ni suppressed growth characteristics such as PH, FW, DW, and LA. However, after addition of NO and SA in growth medium, the growth performance of plant was found to be improved. Under non-stress conditions, application of NO with SA proved superior by giving highest values for these growth parameters. However, application of SA showed statistically similar effect for FW and DW. Under Ni stress, application of NO and SA significantly improved all growth traits relative to the Ni treatment. However, combined application of NO and SA effectively improved these growth characteristics (except LA) in comparison to the alone application of NO and SA.

Physiological and biochemical attributes of plants

The concentrations of Ni, N, P, K, Pro, MDA, Chl *a* and Chl *b* in leaf were significantly affected by the application of NO and SA alone as well as in combination under Ni stress and non-stress conditions (Figs. 2 and 3). The application of NO and SA ameliorated the leaf- Chl *a* and Chl b, N, P, and K content under stress (Figs. 2A and 3A, B). Under non-stress conditions, the highest values for these nutrients content were recorded in plant received both NO and SA together as compared to alone treatment. Similarly, under stress conditions, addition of NO and/or SA to growth medium significantly enhanced leaf- Chl *a* and Chl b, N, P, and K content. The maximum alleviating effect was recorded for content of these nutrients and photosynthetic pigments in plants received NO and SA together as compared to alone application, except leaf-K content. Application of SA improved K content as compared to combined application of NO and SA. Plants grown in medium containing Ni exhibited highest content of Pro content (Fig. 2B). Under Ni stress, combined application of NO and SA was found more effective to induce Pro content in plants than alone treatment. The content of Ni and MDA decreased when plants treated with NO and SA. However, the highest reduction was recorded for MDA and Ni content in leaf with the combined application of NO and SA under non-stress conditions (Figs 2C and 3B). The highest content of MDA in leaf was recorded in plant fed with Ni treatment, while the lowest content was observed with the combined application of NO and SA as compared to alone application. Also, similar increasing trend for Ni content was observed in plants under Ni stress. The activity of CA relative to the control and alone treatment of NO and SA was significantly improved by the combined application of NO and SA, under non-stress conditions (Fig. 4A). However, the highest inhibition in the activity of CA was recorded in plants subjected to Ni. Application of NO and SA together significantly improved the activity of CA as compared to alone application of NO and SA under Ni stress. The data presented in Figure 4B and C reveal that plants subjected to Ni exhibited enhanced activities of antioxidant enzymes, when compared with control plants. However, plants grown in medium containing NO and/or SA showed the highest values for the activities of antioxidant enzymes (SOD, POD and CAT), under Ni stress. The plants supplemented with NO+SA exhibited a significantly improvement for the activities of SOD, POD and CAT, when compared to the control and alone NO and SA–fed plants.

Discussion

The plants grown in medium containing excess Ni concentration exhibited reduced plant growth parameters such PH, FW, DW and LA (Fig.1A-C). These results support

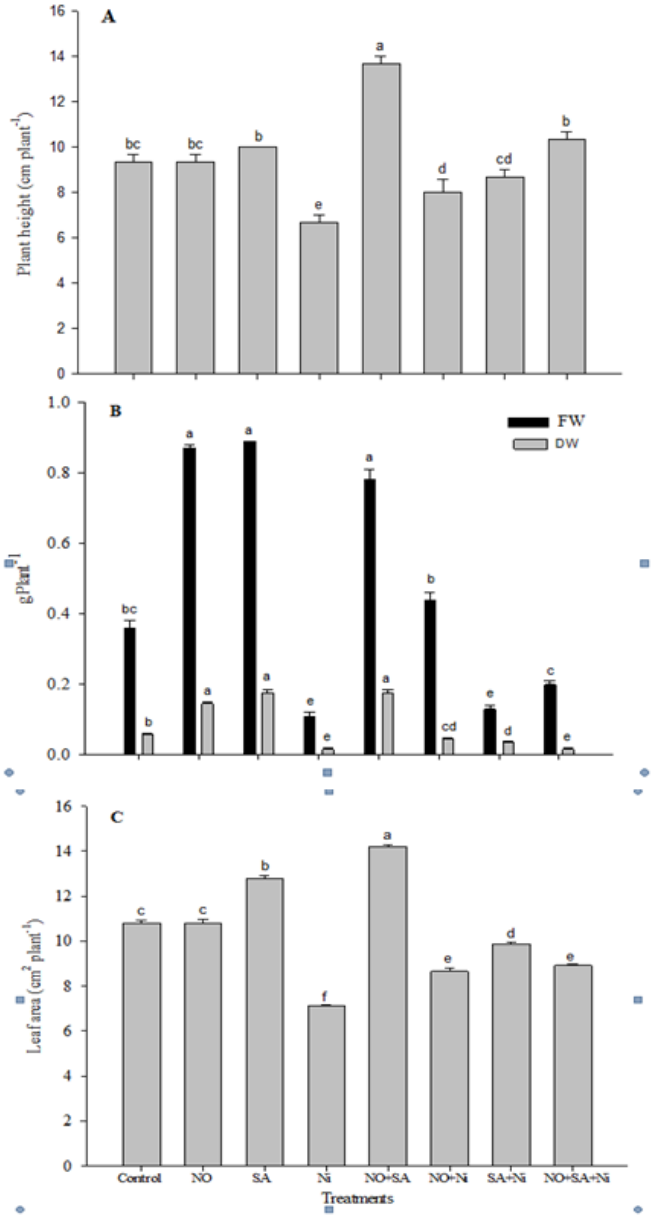

Fig 1. Cumulative effect of NO and SA on Plant height (A), fresh and dry weight (B) and Leaf area (C) of *Triticum aestivum* L. Bars followed by the same letter do not differ statistically at $P<0.05$ (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

the findings of Pandey and Sharma (2002) in cabbage, Gajewska et al. (2006), Gajewska and Skłodowska (2007) and Al-Whaibi et al., (2012) in wheat. The reduction of plant growth in response to Ni stress occurs as a consequence of poor root growth (Moya et al., 1993). The inhibition in plants growth attributes by the Ni stress in wheat plants may be due to poor root growth, reduction of leaf blade area and leaf density (Madhava Rao and Sresty, 2000; Pandey and Sharma, 2002), and disturbed and imbalanced supply of essential nutrients (Palaciosa et al., 1998; Siddiqui et al., 2012b). However, under Ni stress or non-stress conditions, both NO and SA improved growth, but application of both together was found more effective to ameliorate the PH, FW, DW and LA of wheat plants. The enhanced growth might be due to the involvement of NO in better root organogenesis by regulating auxin signaling transduction pathway (Correa-Aragunde et al., 2004). SA enhanced Chl *a* and Chl *b* content in wheat plants (Fig. 2A) that could increase photosynthesis in plants.We postulated that plants subjected to the combined NO and SA application were more tolerant to Ni-stress than those plants fed with alone NO and SA. As we know that photosynthetic pigments such as Chl *a* and Chl *b* play an important role in capturing the light energy for photosynthesis which is responsible for dry matter production of plants. Under nickel stress, both Chl *a* and Chl *b* were severely affected (Fig. 2A). The inhibition of photosynthetic pigments content in plants may be due to the toxic effect of Ni on the photosynthetic apparatus by damaging mesophyll cells, epidermal tissue, thylakoid membrane and chloroplast grana structure (Chen et al., 2009). However, the application of NO and SA alone as well as in combination improved the content of both pigments by mitigating the Ni stress. Laspina et al. (2005) also reported that exogenous application of SNP (NO donor) significantly restored Chl decay induced by cadmium toxicity. An improvement of photosynthetic pigment under Ni toxicity occurred due to the role of NO that protects chloroplast membrane against ROS or involves in Chl metabolic pathway (Lazalt et al., 1997). Also, SA

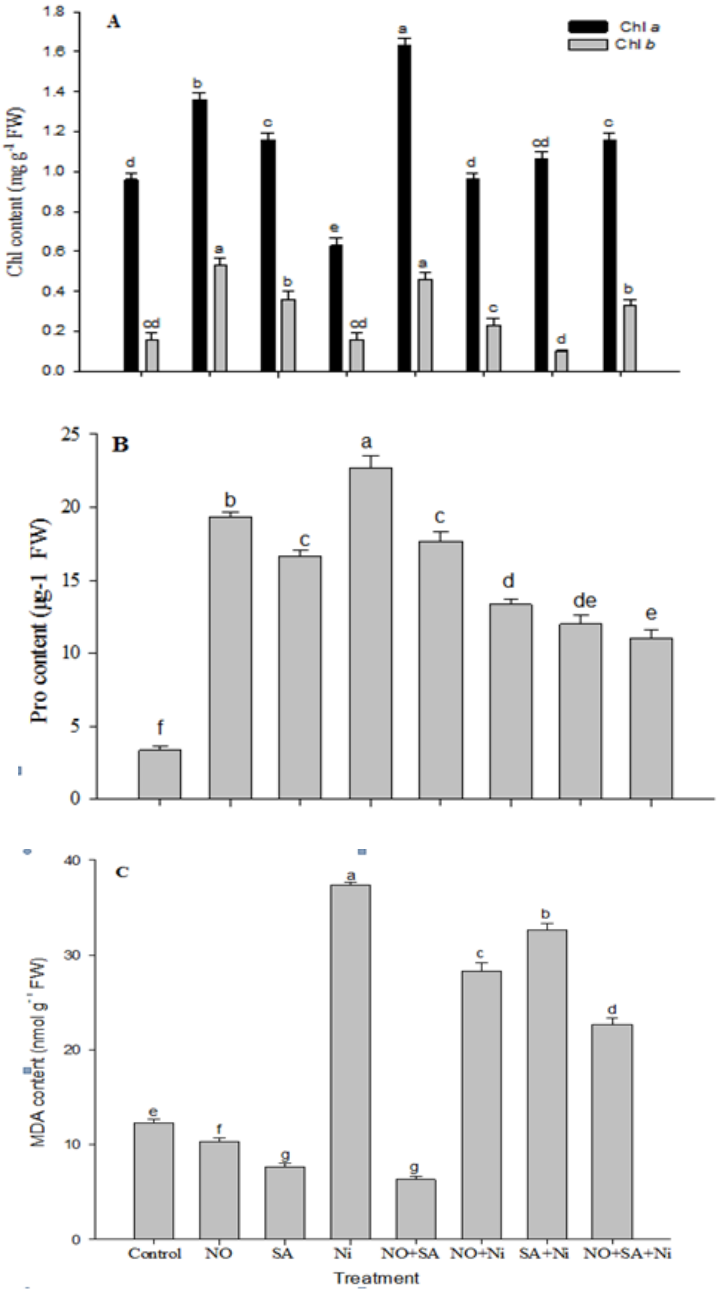

Fig 2. Cumulative effect of NO and SA on the content of Chl a and b (A), Pro (B) and MDA (C) of *Triticum aestivum* L. Bars followed by the same letter do not differ statistically at $P<0.05$ (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

improved Chl *a* and Chl *b* by improving plant growth. Metwally et al. (2003) reported that SA increased the level of tolerance towards high cadmium levels by improving Chl *a* fluorescence parameters. It is well established that plants develop one of the adoptive mechanisms through which plants accumulate Pro to stand under different environmental conditions. Plants grown in Ni containing medium accumulated more Pro than control (Fig. 2B). Stress induced accumulation of Pro was also reported by Al-Whaibi et al. (2012) in wheat and Khan et al. (2012) in mustard. However, plants fed with both NO and SA exhibited highest value for Pro as compared to control plants and Ni-fed plants. An increase in Pro accumulation in plants treated with NO+SA may be due to the accumulation of nutrients (Fig. 3A and B) which elicited a regulatory and stimulatory influence on omsoprotectant synthesis (Pro) (Siddiqui et al., 2012b). Hyper accumulation of Pro may be responsible in alleviating cytoplasmic acidosis and repairing stress-induced damage (Hare et al., 1998). Pro behaves as a signaling molecule to regulate many functions in plants at the level of physiological and molecular, which enable the plant to improve the tolerance to toxicity (Szabados and Savoure, 2010). Under biotic and abiotic stress, MDA is universally accepted as a marker for lipid peroxidation in biological system. An increase in content of MDA in plants grown in medium containing Ni indicates that excess Ni may be responsible for poor plants growth and development by generating reactive oxygen species (ROS) that causes oxidative damage of cell.

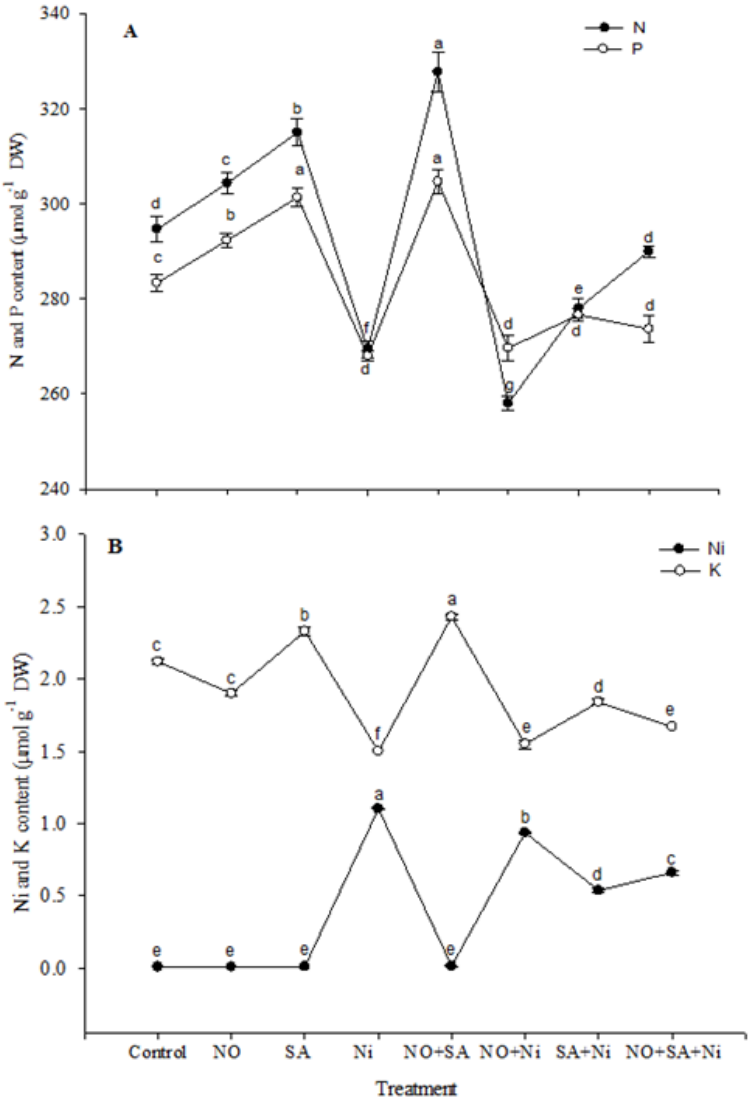

Fig 3. Cumulative effect of NO and SA on the content of N and P (A) and Ni and K (B) of *Triticum aestivum* L. Bars followed by the same letter do not differ statistically at *P<0.05* (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

In the present study, the application of NO and SA alone as well as in combination inhibited the formation of MDA (Fig. 2C). A decrease in the formation of MDA might be due to antioxidant properties of NO that acts as a signaling molecule in activating ROS-scavenging enzymes. Also, NO induces the mitogen-activated protein kinase cascade and enhances expression of defense genes (Durner et al., 1998; Kumar and Klessig, 2000), and inhibits the oxidative damage by regulating the mechanisms of cellular redox homeostasis and transforming O_2^- to H_2O_2 and O_2 (Pandey and Sharma, 2002; Hseu, 2004). Nasir Khan et al. (2012) reported that exogenous application SA increased the tolerance of plants by inhibition of MDA formation in plants. According to Rivas-San Vicente and Plasencia (2011) SA as a true plant hormone improves the defence reaction in plant immunity by coordination with the other hormones such as abscisic acid (ABA), jasmonic acid (JA), gibberellins, ethylene, brassinosteroids, auxins, and cytokinins. Results of this study presented in Figure 3A and B reveal that the concentration of leaf-Ni increased in plants fed with Ni, while other nutrients content such as N, P and K decreased. These results agreed with report of Cataldo et al. (1978) in soybean and Yan et al. (1992) in maize. An increase in Ni content in plants causes poor plant growth and development by affecting growth of root, stem and leaf (Kuznetsov and Shevyakova, 1997; Siddiqui et al., 2011a). However, addition of NO+SA in growth medium containing Ni was found to be more effective in alleviation of Ni stress and improvement of these essential nutrients content (Fig. 3A and B). As we know that balance supply of nutrients to plant results in healthy growth and also alleviates the deleterious effect of abiotic stress. Interestingly, in the present study, an increase in content of N, P and K in plant may be one of the main reasons for improved resistant of wheat plants to Ni stress. Combined application of NO and SA enhanced the content of these elements (N, P and K) resulted in more dry matter production because these essential nutrients play a crucial role in the regulation of physiological and biochemical processes of plants that could help in the detoxification of abiotic stress (Marschner, 2002; Siddiqui et al., 2011a). In plants, CA is a key enzyme that catalyses the reversible inter-conversion of HCO_3^- and CO_2. Plants fed with Ni exhibited a markedly reduction in the

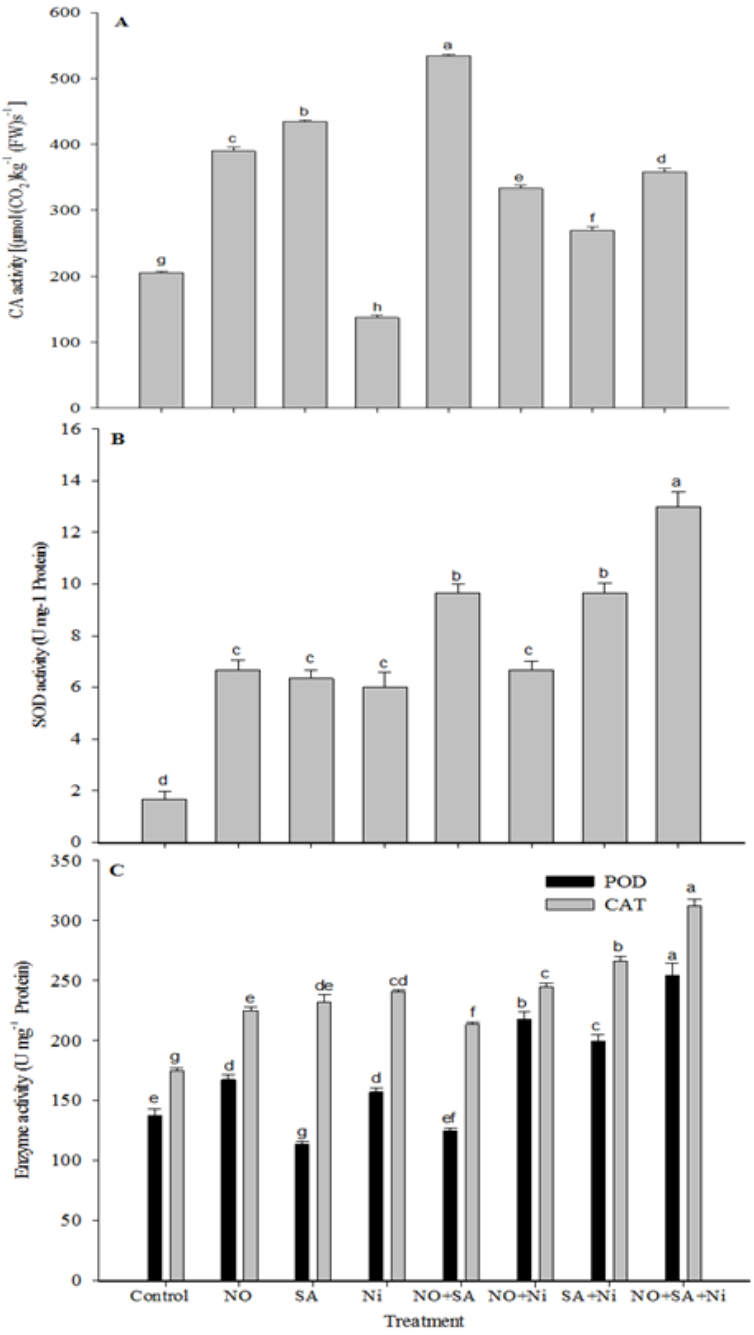

Fig 4. Cumulative effect of NO and SA on the activities of CA (A), SOD (B) and POD and CAT (C) in *Triticum aestivum* L. Bars followed by the same letter do not differ statistically at $P<0.05$ (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

activity of CA (Fig. 4A) and resulted in reduction of photosynthetic activity leading to the poor dry matter production (Fig. 1B). However, application of NO and SA alone as well as in combination induced the activity of CA as compared to control, under stress and non- stress of Ni. An increase in the activity of CA enzyme was reported by Al-Whaibi et al. (2012) in wheat with SA application, and by Khan et al. (2012) in mustard with NO application. The enhanced activity of CA due to the application of NO and SA could be involved in several physiological processes such as acid base balance, CO_2 transfer, ion exchange, respiration, and photosynthetic CO_2 fixation (Tiwari et al., 2005), which may be one of the reason behind the improved resistance of wheat plants to Ni stress. Under stress, it is well known that the chances of survival or death of plant depend on the normal physiological functions that need an effective destruction of generated ROS through the induction of antioxidant enzymes (Srivastava et al., 2006; Siddiqui et al., 2012b). In the present experiment, we observed that application of NO and SA was found to be effective in restoration of altered plant growth by improving the activities of antioxidant enzymes (SOD, CAT and POS) (Fig. 4B and C). On the other hand, the antioxidative enzymes restored their maximum activities and showed a remarkable increase when Ni-stressed plants were treated with SA alone or in combination with NO. The restoration of altered metabolic functions induced by Ni-toxicity may be due to role of NO in activation of gene responsible for abiotic tolerance (Xu et al., 2011), and in detoxification of ROS either with direct interaction with superoxide (Nakazawa et al., 1996) or may

enhance the antioxidant capacity of cell by increasing the activities of antioxidant enzymes (Hsu and Kao, 2004; Khan et al., 2012). Also, the ameliorating effect can be explained on the basis of role of SA acting as a signaling molecule responsible for inducing H_2O_2 and other ROS which act as secondary messengers that express defence related genes associated with systematic acquired resistance in several plant species (Chen et al., 1993; Rivas-San Vicente and Plasencia, 2011).

Materials and methods

Plant materials, cultures and treatments

To achieve the objective of the present experiment, the response of wheat cultivar Samma to NO and SA under Ni stress was evaluated by conducting a growth chamber experiment at Department of Botany and Microbiology, King Saud University, Riyadh. Healthy and uniformed seeds of wheat (*Triticum aestivum* L. cv. Samma) (Collected from local market of Riyadh) were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with sterilized double distilled water (DDW). The seeds were sown in plastic pots (4 cm diameter), filled with perlite, supplied with Raukura's nutrient solution (Smith et al., 1983) and were allowed to germinate in growth chamber. The pots were arranged in a simple randomized design in greenhouse with a single factor and 4 replicates. The experimental pots were irrigated at every two days with DDW (100 mL) to keep the perlite moist. The treatments were given as follows: (i) Ni_0 mM + NO_0 mM+ SA_0 mM (control), (ii) Ni_1 mM + NO_0 mM+ SA_0 mM, (iii) Ni_0 mM + $NO_{0.5}$ mM+ SA_0 mM, (iv) Ni_0 mM + NO_0 mM+ $SA_{0.5}$mM, (v) Ni_1 mM + $NO_{0.5}$ mM+ SA_0 mM, (vi) Ni_1 mM + NO_0 mM+ $SA_{0.5}$ mM, (vii) Ni_0 mM + $NO_{0.5}$ mM+ $SA_{0.5}$ mM, (viii) Ni_1 mM + $NO_{0.5}$ mM+ $SA_{0.5}$ mM. The sources of Ni and NO were nickel chloride and sodium-nitroprusside (SNP) respectively.. The plants were sampled at 30 days, after sowing to assess their growth characteristics [fresh weight (FW) plant^{-1}, dry weight (DW) plant^{-1}, plant height (PH) plant^{-1} and leaf area (LA) leaf^{-1}] and physiological and biochemical parameters [photosynthetic pigments (chlorophyll (chl *a* and Chl *b*), concentrations of Ni^{2+}, potassium (K^+), nitrogen (N), phosphorus (P), proline (Pro) and malondialdehide (MDA), and activities of carbonic anhydrase (CA), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD).

Measurement of plant Growth Characteristics

Plant height was recorded using a meter scale after removal of plants from the pots of each treatment. After recording FW with balance, plants were placed in a 60°C oven for 48 h and then were weighed for DW. Leaf area (LA) was measured by Leaf area meter (LI.COR-model LI-3000).

Determination of physiological and biochemical parameters

Chlorophyll (Chl) was extracted from fresh leaves using the DMSO method of Barnes et al. (1992).

To determine Ni^{2+} concentration, the digestion approach of Zheljazkov and Nielson (1996) as modified by Hseu (2004) was followed using an atomic absorption spectrometer (Model iCAP6000, Thermo-Scientific, England). Leaf K content was estimated by flame spectroscopy. Leaf N and P contents were determined according to the method of Lindner (1944) and Fiske and Subbarow (1925), respectively. Proline concentration was determined spectrophotometrically by adopting the ninhydrin method of Bates et al. (1973) using L-proline as a standard. Malondialdehyde (MDA) content in leaves was determined according to the method of Heath and Packer (1968).

Enzyme Activity

To determine the enzymatic activities of the antioxidant proteins, a crude enzyme extract was prepared by homogenizing 500 mg of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was used for the enzymatic assays described below. All enzyme activities were expressed as unit mg^{-1} protein min^{-1}. The method of Chance and Maehly (1955) was used to determine POD (EC: 1.11.1.7) activity. A unit of peroxidase activity was the amount of purpurogallin formed per mg protein per minute. Aebi (1984) method was used to measure CAT (EC: 1.11.1.6) activity. The activity of SOD (EC: 1.15.1.1) was determined the nitro blue tetrazolium (NBT) method (Giannopolitis and Ries, 1977). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction. The activity of CA (EC: 4.2.1.1) activity was determined by the method of Dwivedi and Randhawa (1974). The results were expressed as [µmol (CO_2) kg^{-1} (FW) s^{-1}.

Statistical Analysis

Each pot was treated as one replicate and all the treatments were repeated four times. The data were analysed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's Multiple Range Test (DMRT) at the p<0.05 % level.

Conclusion

In conclusion, the results of the present study reveal that application of NO with SA was found more effective than alone application of NO and SA. The combined application of NO and SA proved beneficial to suppress the inhibitory effect of Ni-toxicity by stimulating the activities of enzymes (CA, SOD, POD and CAT) and accumulation of osmoprotectant (Pro) and essential nutrients. An increase in the activity of antioxidant enzymes and the accumulation of essential nutrients and Pro may be one the reasons for reduction of MDA accumulation and improvement of photosynthetic pigments (Chl *a* and Chl *b*) resulted in a better plant growth and development.

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