TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2013) 37: 1155-1165
© TÜBİTAK
doi:10.3906/bot-1301-17

Protective role of foliar-applied nitric oxide in *Triticum aestivum* under saline stress

Farhana KAUSAR[1], Muhammad SHAHBAZ[1,*], Muhammad ASHRAF[2]
[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]University College of Agriculture, University of Sargodha, Sargodha, Pakistan

Received: 08.01.2013 • **Accepted:** 21.07.2013 • **Published Online:** 30.10.2013 • **Printed:** 25.11.2013

Abstract: A study was conducted to assess whether foliar-applied nitric oxide (NO) could alleviate the adverse effects of salt stress on wheat (*Triticum aestivum* L.). Four sodium nitroprusside levels (control [water spray] and 0.05, 0.10, and 0.15 mM) were sprayed as a donor of NO on the leaves of cultivar S-24 plants grown under nonsaline and saline conditions (150 mM NaCl). Data for growth and yield, chlorophyll contents, activities of antioxidants, and concentrations of mineral nutrients were recorded. Root-medium salinity adversely affected shoot and root dry weight, shoot length, and yield attributes of the wheat plants while it enhanced the activities of antioxidants, proline accumulation, and concentrations of shoot and root Na^+ and Cl^-. Foliar-applied NO improved growth of only nonstressed plants. Exogenously applied NO enhanced the activities of antioxidant enzymes (superoxide dismutase [SOD], peroxidase [POD], and catalase [CAT]) and levels of soluble proteins and proline, in both stressed and nonstressed wheat plants. Overall, exogenous application of NO enhanced chlorophyll contents; activities of CAT, POD, and SOD; and levels of soluble proteins and total free proline in the salt stressed wheat plants. The exogenous application of NO had a protective role against salt-induced oxidative damage by enhancing the activities of antioxidant enzymes, thereby improving plant growth under saline stress.

Key words: Wheat, nitric oxide, salinity, antioxidants, proline

1. Introduction

Salinity is one of the most damaging factors responsible for reduction in plant growth and yield (Shahbaz et al., 2011, 2012; Adebooye et al., 2012; Perveen et al., 2012a). Salinity stress perturbs a number of metabolic processes, including photosynthesis (Shahbaz et al., 2012; Hameed et al., 2013). Salinity also causes the generation of oxidative stress in plants by producing a variety of reactive oxygen species (ROS) like superoxide radical ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH^-$) (Joseph and Jini, 2010). Oxidative stress is generally a limiting factor for plant growth and productivity. However, plants protect themselves from the oxidative damage by developing an efficient defense system comprising enzymatic and nonenzymatic antioxidant enzyme systems. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and glutathione peroxidase (GPOD) (Joseph and Jini, 2010), and they can scavenge ROS produced in plants under saline conditions (Batool et al., 2012). This scavenging depends upon the sequential and simultaneous action of antioxidant enzymes (Shahbaz et al., 2008). Under saline conditions, accumulation of compatible organic osmolytes such as soluble sugars and free proline is also enhanced in most plants (Perveen et al., 2011; Ashraf et al., 2012; Perveen et al., 2012b). Under salt stress, Na^+ and Cl^- concentrations in plant tissues are increased over the demand of the crops, which cause adverse effects on plants (Shahbaz and Zia, 2011; Shahbaz and Ashraf, 2013). Excessive external supply of Na^+ causes low K^+/Na^+ ratios in plants exposed to salt stress (Naheed et al., 2008; Shahbaz et al., 2013). Furthermore, yield attributes decrease substantially under saline regimes (Kanwal et al., 2011).

A variety of chemicals, both organic and inorganic, are currently being used as exogenous applications to plants exposed to stressful conditions, and in most cases spectacular results in terms of improved growth and yield have been shown (Farooq et al., 2009). Nitric oxide (NO), a small signaling molecule, has been reported as very effective in playing a significant role in plant defense against a number of stresses (Hasanuzzaman et al., 2012). Nitric oxide is effective in enhancing growth and development from seed germination to the adult stage (Desikan et al., 2004). It also promotes flowering and hormonal responses (Zhou et al., 2005). However, exogenous application of NO has been reported to enhance relative water content, chlorophyll, and proline contents

* Correspondence: shahbazmuaf@yahoo.com

of wheat plants under arsenic-induced oxidative stress (Hasanuzzaman et al., 2012). Pretreated wheat seedlings with NO had high ascorbate and reduced glutathione contents and activities of monodehydroascorbate reductase, dehydroascorbate reductase, and glyoxalase I and II under saline stress (Hasanuzzaman et al., 2011) or high temperatures (Hasanuzzaman et al., 2012). The role of NO in plants is highly diverse because of its involvement in different physiological mechanisms (Ruan et al., 2004). Foliar spray of NO has been reported to improve growth. It also enhances chlorophyll contents (Ruan et al., 2004) and its exogenous application protects plants from oxidative damage by enhancing antioxidant system capacity (Tuncz-Ozdemir et al., 2009). It regulates plant growth and development starting from seed germination to maturity (Jin et al., 2009). It is effective in enhancing root dry weight in maize seedlings and activities of SOD and CAT in the roots of *Lupinus luteus* under saline stress (Zhang et al., 2006). It is believed that NO is involved in modulation of ROS and enhancement of antioxidant enzymes under various abiotic stresses (Uchida et al., 2002) including saline stress. It acts as a signaling molecule to sense the sugars (Chen et al., 2009). Exogenous application of NO improves plant K^+ contents while decreasing Na^+ concentration, thereby maintaining the K^+/Na^+ ratio in plants under salt stress (Zheng et al., 2009).

Considering the role of NO in plant growth, it was investigated whether or not foliar application of NO could be effective in improving growth and physiological activities in wheat plants under saline conditions. Thus, the main objectives of the present study were to observe NO-induced modulation in growth, activities of antioxidant enzymes, accumulation of proline and glycinebetaine, ionic homeostasis, and yield attributes of wheat under saline stress.

2. Materials and methods

The present investigation was conducted to elucidate the role of exogenously applied NO on wheat (*Triticum aestivum* L.) grown under saline conditions. The experiment, using wheat cultivar S-24, was conducted in a naturally lit net-house in the Old Botanical Garden of the University of Agriculture, Faisalabad, Pakistan. The average day and night temperatures were 31.4 ± 5 °C and 26.4 ± 4 °C, respectively. The relative humidity ranged from 30.8% to 63.7%, and day length ranged from 11.5 to 12.5 h. The seed of cultivar S-24 was obtained from the Botany Department of the University of Agriculture, Faisalabad. Fifteen seeds were directly sown into plastic pots (23 cm in diameter and 26 cm in length) containing 10 kg of well-washed river sand. After the germination of the seeds, the seedlings were thinned to 6 plants per pot. The experiment was laid down in a completely randomized design with 4 replications. There were 2 salt regimes, i.e. control (full-strength Hoagland's nutrient solution) and 150 mM NaCl in full-strength Hoagland's nutrient solution. Salt stress was applied after 57 days of sowing and the desired level of 150 mM of NaCl was developed step-wise by adding a 50 mM aliquot to each pot every day. Four levels of sodium nitroprusside (control [water spray] and 0.05, 0.10, and 0.15 mM) were foliar-applied as the NO donor 1 week after the start of salt treatment. Tween-20 as a 0.1% solution was used as a surfactant to ensure maximum absorption. After 2 weeks of NO application, the data for various attributes were recorded. Two plants from each replicate were uprooted carefully, washed with distilled water, and had the lengths of both shoot and root recorded separately. After measuring shoot and root lengths, plants were oven-dried at 65 °C for 72 h and their dry mass was recorded.

2.1. Photosynthetic pigments

Chlorophyll *a* and *b* contents were determined using the method of Arnon (1949). Fresh leaf (100 mg) material was extracted in 80% acetone overnight and centrifuged at 10,000 × *g* for 5 min. The optical densities of the filtrates were then measured on a spectrophotometer (Hitachi U-2001, Tokyo, Japan) at 645 and 663 nm.

2.2. Free proline determination

For the determination of free proline, the Bates et al. (1973) method was used. Fresh leaf material (0.5 g) was extracted in 10 mL of 3% sulfosalicylic acid and filtered. Then 2 mL of the extract was mixed with 2 mL of acid ninhydrin solution and 2 mL of glacial acetic acid. All samples were incubated at 100 °C for 60 min and cooled in an ice bath. Next, 4 mL of toluene was added to the blend by mixing it vigorously. The chromophore, containing toluene, was aspirated and the absorbance was measured at 520 nm on a spectrophotometer. The proline concentration was determined using a standard curve and calculated on a fresh mass basis.

2.3. Glycinebetaine determination

Glycinebetaine was determined from fresh leaves following the method of Grieve and Grattan (1983) with some modifications. Fresh leaf material (0.5 g) was ground in 10 mL of distilled water and filtered. After filtration, 1 mL of the filtrate was mixed with 1 mL of 2N HCl. Next, 0.5 mL of this mixture was taken in a glass tube and mixed with 0.2 mL of potassium triiodide solution. The contents were shaken and cooled in an ice bath for 90 min with occasional shaking, and then 2.0 mL of ice-cooled distilled water and 9 mL of 1,2-dichloromethane (cooled at –10 °C) were added to the mixture. The 2 layers formed in the mixture were mixed by passing a continuous stream of air for 1–2 min while tubes were still in an ice bath (4 °C). The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm with a spectrophotometer. The concentrations of the betaine were calculated against a standard curve.

2.4. Antioxidant activities

Leaf fresh material (0.5 g) was ground in 10 mL of cooled phosphate buffer (pH 7.8) in an ice bath. The extract was filtered and centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatant was used for the determination of activities of antioxidant enzymes SOD, POD, and CAT and total concentration of soluble proteins.

2.4.1. Superoxide dismutase

The activity of SOD was determined following Giannopolitis and Ries (1977) on the basis of inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture (3 mL) contained 50 µL of enzyme extract along with the sample, with 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA, and 50 mM phosphate buffer (pH 7.8). The cuvettes containing samples were illuminated under the light of 15 W fluorescence lamp for 15 min. The absorbance of the irradiated solution was recorded at 560 nm using a UV-visible spectrophotometer (Hitachi U-2001).

2.4.2. Peroxidase and catalase

Activities of CAT and POD were determined following the method of Chance and Maehly (1955) with slight modification. The POD reaction solution (3 mL) consisted of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2, and 100 µL of the enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20 s. The CAT reaction solution (3 mL) was a combination of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2, and 100 µL enzyme extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were recorded sequentially after every 20 s.

2.5. Determination of total soluble proteins

Total soluble proteins were determined using the method of Bradford (1976). Pure bovine serum albumin was used as a standard.

2.6. Determination of mineral nutrients

The dried ground shoot or root material (0.1 g) was digested with sulfuric acid and hydrogen peroxide following Wolf (1982).

2.6.1. Determination of Na^+, Ca^{2+}, and K^+

A flame photometer (Jenway PFP-7) was used for determining shoot and root Na^+, Ca^{2+}, and K^+ from the above digested samples. A series of standard solutions of each of the above nutrients (Na^+, Ca^{2+}, and K^+) were used to determine the ion concentration.

2.6.2. Cl^- determination

For the determination of Cl^-, the ground shoot or root material (100 mg) was extracted in 10 mL of distilled water at 80 °C for 4 h. The Cl^- content in the extracts was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

2.7. Yield parameters

Yield attributes including seed yield per plant (g), 100-seed weight (g), and number of seeds per plant were recorded at crop maturity.

2.8. Statistical analysis

Data were recorded and analyses of variance for all the parameters were computed using the COSTAT computer package (Cohort Software Berkeley, CA, USA). The least significant differences between means were calculated (Snedecor and Cochran, 1989).

3. Results

Shoot and root dry weights decreased significantly due to imposition of root-medium saline stress. Foliar-applied various levels of NO slightly enhanced shoot dry weight under nonsaline conditions, while such effect was not so pronounced under saline stress. Nitric oxide did not cause a significant effect on root dry weight under either saline or nonsaline conditions (Table 1; Figures 1A and 1B).

The imposition of salt stress markedly reduced shoot and root lengths of the wheat plants. Foliar-applied NO increased shoot length under nonsaline conditions, while the reverse was true in the case of root length under both nonstressed and salt-stress conditions. Application of NO at 0.05 or 0.1 mM proved to be effective in enhancing shoot length under saline conditions (Table 1; Figures 1C and 1D); however, the higher concentration of NO did not prove so effective.

Exogenous application of NO as foliar spray and root-medium saline stress did not affect chlorophyll *a* and *b* contents and chlorophyll *a*/*b* ratio of hexaploid wheat (Table 1; Figures 1E, 1F, and 1G).

The imposition of salt stress (150 mM) did not alter total soluble proteins in the wheat plants. Exogenously applied NO significantly increased soluble proteins in wheat cultivar S-24 under both saline and nonsaline regimes. Of various foliar-applied NO levels, 0.05 mM caused the maximum increase in soluble proteins under saline conditions, and 0.10 mM under both saline and nonsaline conditions (Table 1; Figure 1H).

The activity of SOD was found to be increased under salt stress. Exogenously applied varying levels of NO were found to be effective in enhancing the SOD activity in the wheat plants under both saline and nonsaline conditions. Of various foliar-applied levels of NO, 0.05 mM was proven to be very effective in increasing the SOD activity under saline stress (Table 1; Figure 2A).

The activity of CAT increased markedly in the salt stressed wheat plants. However, foliar-applied NO did not alter the CAT activity under saline or nonsaline conditions (Table 1; Figure 2B). Root-medium applied salt stress slightly increased the activity of POD, which was further increased by the exogenous application of NO. Enhancement in the

Table 1. Mean squares from analyses of variance of data for growth attributes, chlorophyll contents, and biochemical analysis of *Triticum aestivum* plants foliar-sprayed with nitric oxide under nonsaline or saline conditions.

Source of variance	df	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Chl *a* (mg g^{-1} fresh weight)
Salinity (S)	1	9.717***	0.142***	1798.35***	1798.3***	0.00033 ns
NO treatment	3	0.773***	0.0280 ns	148.37**	148.37**	0.0437 ns
S × NO	3	0.523***	0.011 ns	82.097*	82.097*	0.066 ns
Error	24	0.045	0.141	26.182	26.182	0.098
Source of variance	df	Chl *b* (mg g^{-1} fresh weight)	Chl *a/b*	SOD (units mg^{-1} protein)	CAT (units mg^{-1} protein)	POD (units μg^{-1} protein)
Salinity (S)	1	0.0093 ns	0.243 ns	773.7**	0.0017**	205.03*
NO treatment	3	0.0332 ns	0.1964 ns	1532.1***	3.738 ns	857.3***
S × NO	3	0.0225 ns	0.011 ns	344.8**	1.622 ns	34.498 ns
Error	24	0.0123	0.1863	67.957	1.468	47.44
Source of variance	df	Soluble proteins (mg g^{-1} fresh weight)	Free proline (μmol g^{-1} fresh weight)	Glycinebetaine (μmol g^{-1} fresh weight)		
Salinity (S)	1	0.0062 ns	153126.06***	1312.9 ns		
NO treatment	3	0.159***	3595.6 ns	6279.3*		
S × NO	3	0.103 **	2978.8 ns	817.3 ns		
Error	24	0.014	1664.7	1859.7		

*, **, and *** = significant at 0.05, 0.01, and 0.001 levels respectively; ns = nonsignificant.
Chl = Chlorophyll; df = degrees of freedom; SOD = superoxide dismutase; POD = peroxide dismutase; CAT = catalase.

activity of POD by NO was uniform under both nonstressed and salt-stress conditions (Table 1; Figure 2C). Overall, application of NO at 0.05 or 0.10 mM was more effective in enhancing the enzyme activities in the wheat plants as compared to the other NO levels used (Table 1).

Free proline content of wheat cultivar S-24 increased significantly under salt stress conditions. However, foliar application of varying levels of NO did not alter proline content under either control or saline stress (Table 1; Figure 2D). Salinity of the growth medium did not show any marked effect on glycinebetaine in wheat cultivar S-24. However, exogenously applied NO at 0.05 mM slightly enhanced glycinebetaine concentration under the saline regimes (Table 1; Figure 2E).

Exogenous application of NO as foliar spray markedly increased yield per plant, number of seeds per plant, and 100-seed weight of wheat cultivar S-24 under nonstressed conditions, while only 0.1 mM NO enhanced grain yield per plant under saline conditions. All other NO levels did not show significant effect on yield attributes of wheat under saline conditions (Table 2; Figures 2F, 2G, and 2H).

Salt stress applied through the rooting medium significantly increased shoot Na^+ in the wheat plants. Exogenous application of varying levels of NO caused a slight decrease in shoot Na^+ of wheat cultivar S-24 under nonsaline conditions and an increase under saline stress (Table 2; Figure 3A). Root Na^+ increased significantly due to saline stress. Foliar-applied NO enhanced root Na^+ contents in the wheat plants under the saline regimes (Table 2; Figure 3B).

Imposition of salinity through the root growing medium did not alter either shoot or root Ca^{2+} contents, and exogenous application of different levels of NO also had nonsignificant effects on the Ca^{2+} contents in both tissues (Table 2; Figures 3C and 3D).

Root-medium salt stress slightly decreased shoot K^+ contents, while it did not alter root K^+ contents of wheat plants. However, exogenous application of varying levels of NO, except 0.15 mM, slightly decreased shoot K^+ contents under both saline and nonsaline regimes, while it increased root K^+ contents under both nonsaline and saline regimes. The highest level of NO (0.15 mM) showed a nonsignificant effect on both shoot and root K^+ under saline conditions (Table 2; Figures 3E and 3F).

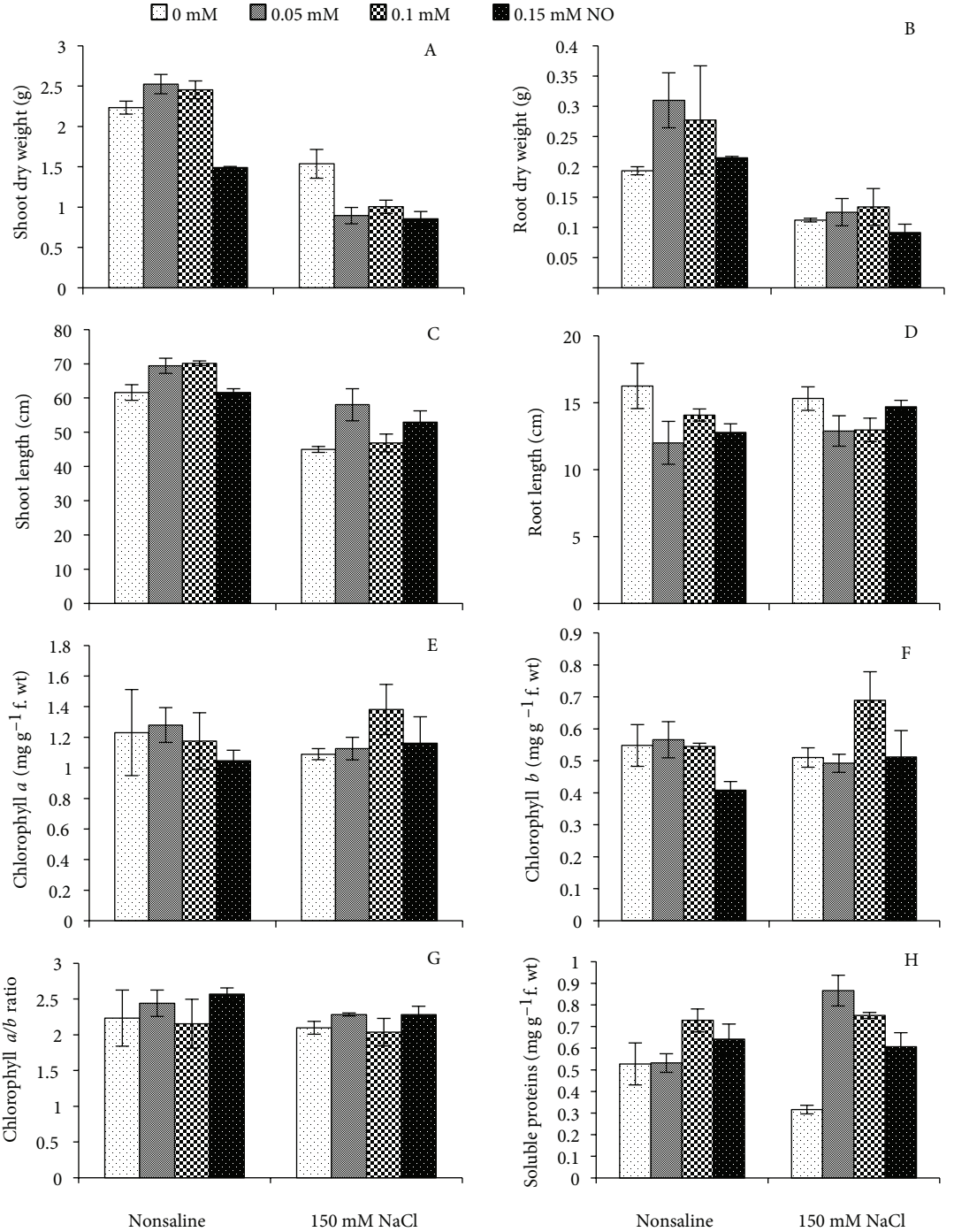

Figure 1. Growth attributes, chlorophyll contents, and soluble proteins of *Triticum aestivum* plants foliar-sprayed with NO under nonsaline or saline conditions.

Salt stress applied through the rooting medium significantly increased shoot and root Cl^- contents of wheat cultivar S-24. However, exogenous application of varying levels of NO caused a slight decrease in shoot Cl^- under nonstressed and root Cl^- under salt-stress conditions (Table 2; Figures 3G and 3H).

4. Discussion

Exogenous application of NO can be used to improve crop growth under various abiotic stresses (Zhang et al., 2006; Zheng et al., 2009; Hasanuzzaman et al., 2011, 2012), because it acts as a signaling molecule involved in enhancing growth of various crops. In the present study,

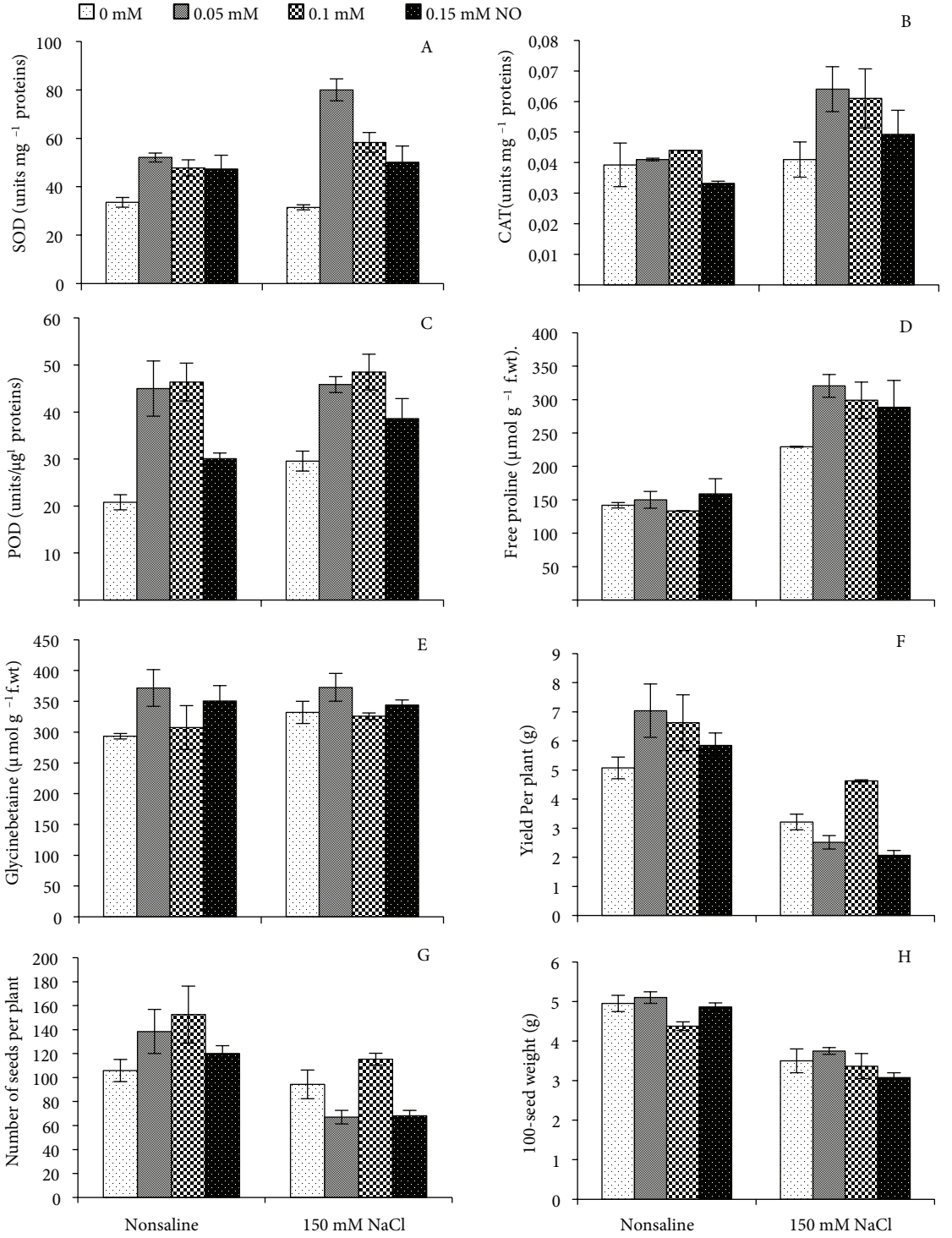

Figure 2. Activities of antioxidants, free proline, glycinebetaine, and yield attributes of *Triticum aestivum* plants foliar-sprayed with NO under nonsaline or saline conditions.

saline stress significantly reduced the growth of wheat plants, which is in agreement with some previous reports on many crops such as sunflower (Akram et al., 2012), wheat (Perveen et al., 2010), cotton (Shaheen et al., 2012), and canola (Saadia et al., 2012). However, foliar-applied NO enhanced the growth of wheat cultivar S-24 under nonstressed conditions only, which was in agreement with the findings of Kausar and Shahbaz (2013). Foliar-

Table 2. Mean squares from analyses of variance of data for mineral nutrition and yield attributes of *Triticum aestivum* plants foliar-sprayed with nitric oxide under nonsaline or saline conditions.

Source of variance	df	Shoot Na^+ (mg g^{-1} dry weight)	Shoot Ca^{2+} (mg g^{-1} dry weight)	Shoot K^+ (mg g^{-1} dry weight)	Shoot Cl^- (mg g^{-1} dry weight)	Root Na^+ (mg g^{-1} dry weight)
Salinity (S)	1	1738.1***	1.759 ns	14.231*	1505.6***	359.59***
NO treatment	3	206.51 ns	76.76 ns	21.214***	87.72**	205.27***
S × NO	3	362.12*	2.799 ns	6.4678*	108.34**	18.071**
Error	24	89.344	34.57	2.0903	15.86	2.550
Source of variance	df	Root Ca^{2+} (mg g^{-1} dry weight)	Root K^+ (mg g^{-1} dry weight)	Root Cl^- (mg g^{-1} dry weight)	Yield per plant (g)	Number of seeds per plant
Salinity (S)	1	34.77 ns	11.08 ns	108.41***	73.894***	14,836.8***
NO treatment	3	88.97 ns	72.578***	4.962**	4.536*	2544.6*
S × NO	3	168.28 ns	12.904*	4.424**	3.4894*	1274.7 ns
Error	24	111.06	3.443	0.877	1.112	627.1
Source of variance	df	100-seed weight (g)				
Salinity (S)	1	15.68***				
NO treatment	3	0.5041*				
S × NO	3	0.206 ns				
Error	24	0.1503				

*, **, and *** = significant at 0.05, 0.01, and 0.001 levels respectively; ns = nonsignificant; df = degrees of freedom.

applied NO caused improvement in growth in maize (Zhang et al., 2004) and rice (Uchida et al., 2002), both under nonstressed and salt stress conditions. Exogenous application of NO was earlier reported to enhance salinity tolerance in wheat seedlings (Hasanuzzaman et al., 2011).

In the present study, salt stress did not affect chlorophyll contents, which is in agreement with the findings of Shahbaz et al. (2011), who observed a nonsignificant effect of saline stress on chlorophyll contents in sunflower. In contrast, a salt-induced adverse effect on chlorophyll contents has been observed in various species like rice (Shahbaz and Zia, 2011) and wheat (Shahbaz et al., 2008). Exogenous application of NO has been reported to effectively reduce salt-induced ion toxicity by protecting the membrane of the cell organelle containing chlorophyll, as earlier observed in many crops like rice, lupin, and maize (Uchida et al., 2002; Wu et al., 2010). Although NO application was found effective in enhancing chlorophyll contents in wheat plants (Ruan et al., 2004), such a prominent effect was not observed in our study. This might have been due to many factors, such as the type of wheat cultivar, NO concentration, and time of application used (Wu et al., 2010). Nitric oxide-induced high accumulation of chlorophyll was also observed in wheat under heat stress (Hasanuzzaman et al., 2012) and arsenic-induced oxidative stress (Hasanuzzaman and Fujita, 2013).

Under salt stress, plants are adversely affected due to oxidative damage caused by ROS. These reactive species are scavenged by a variety of enzymatic and nonenzymatic antioxidants (Apel and Hirt, 2004). Our findings show that salt stress increased the activities of CAT, SOD, and POD, which is in agreement with the previous findings on wheat (Ashraf et al., 2012) and sunflower (Noreen and Ashraf, 2008). Nitric oxide has been reported to be actively involved as a signaling molecule in scavenging ROS by regulating/enhancing the activities of antioxidant enzymes like CAT, SOD, and POD (Wang et al., 2004). In our study, application of NO as foliar spray markedly increased the activities of SOD, POD, and CAT and total soluble proteins, which was in agreement with some previous studies on different crops such as cucumber (Fan et al., 2007), rice (Wang et al., 2004), and beans (Tuncz-Ozdemir et al., 2009) in which the activities of SOD, POD, and CAT were reported to be increased due to NO application. Nitric oxide-induced enhanced activity of CAT has been reported under various abiotic stresses (Hasanuzzaman et al., 2011). In addition, application of NO was reported to enhance the activities of monodehydroascorbate

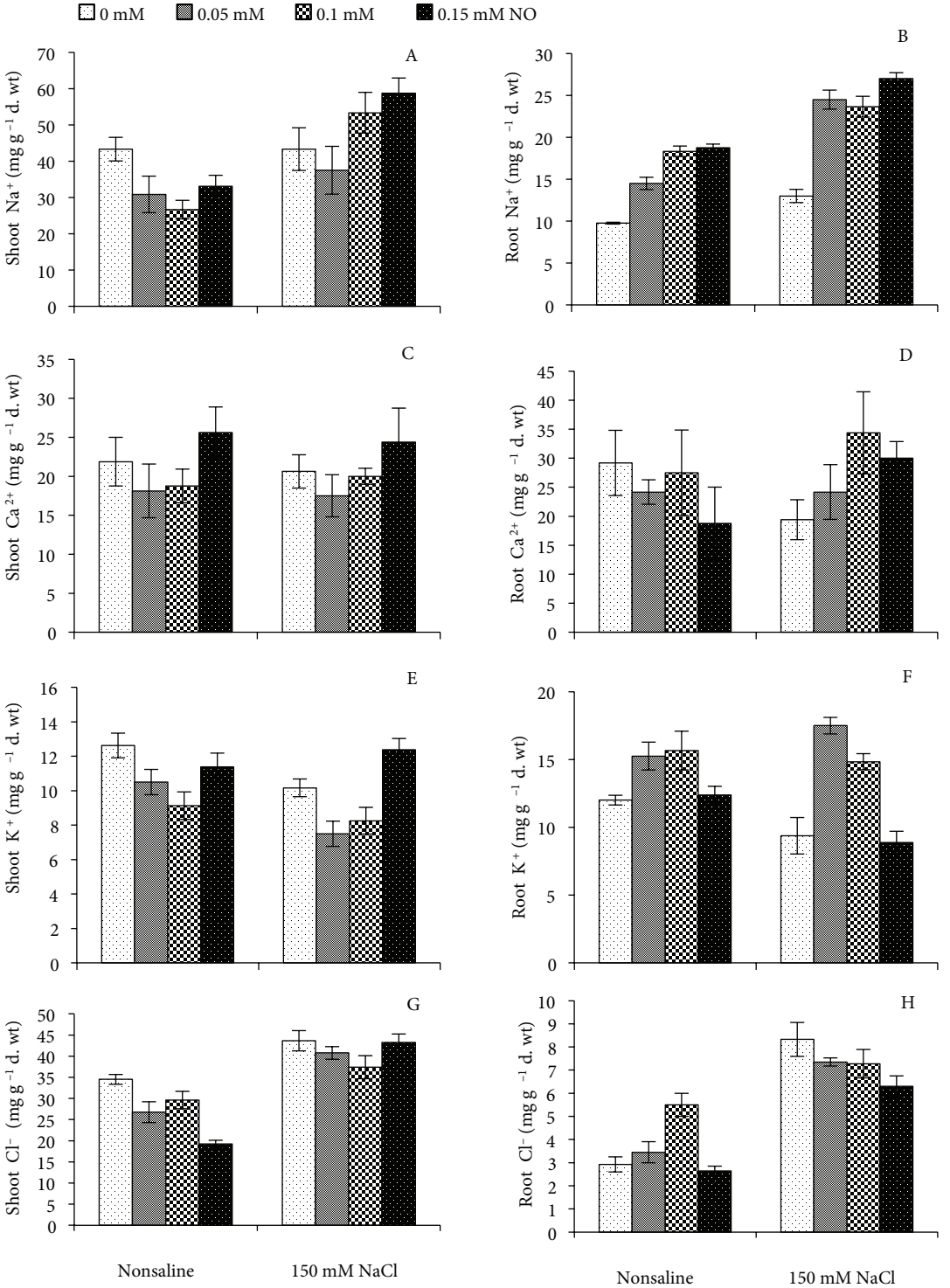

Figure 3. Shoot and root mineral nutrients of *Triticum aestivum* plants foliar-sprayed with nitric oxide under nonsaline or saline conditions.

reductase, dehydroascorbate reductase, glutathione reductase, glutathione peroxidase, and glyoxalase I and II of wheat under saline stress (Hasanuzzaman et al., 2011). A similar type of NO effect was also observed in wheat under arsenic-induced oxidative stress (Hasanuzzaman and Fujita, 2013) or heat stress (Hasanuzzaman et al.,

2012). However, the effectiveness of NO is dependent on its time of application, stage of development at which NO application or stress is applied to plants, type of variety, and environmental conditions (Wu et al., 2010). Soluble proteins play an important role in plant metabolism by acting as typical osmoprotectants and a signal molecule in sugar signaling and sensing systems (Chen et al., 2009). Furthermore, foliar-applied NO accelerates accumulation of soluble proteins in plants (Zanardo et al., 2005). A positive correlation between SOD and soluble proteins (r = 0.8486***) has been observed in our experiment, which indicates that SOD activity increased with an increase in soluble proteins, and high SOD activity was observed due to application of NO. Such an elevated SOD activity was also observed in rice and wheat when NO was applied at the seedling stage (Zheng et al., 2009).

One of the reasons for improved stress tolerance by exogenous application of NO could be due to high accumulation of proline in plants (Zhu, 2002). Exogenous treatment of NO activates some key enzymes involved in the synthesis of proline (Zhang et al., 2008) and more proline accumulation has been noted under salt stress than NO application alone (Zhang et al., 2009). In our study, wheat plants accumulated proline in response to salinity, and foliar-applied NO further increased its accumulation. Nitric oxide-induced increase in proline accumulation has already been observed in wheat under arsenic-induced oxidative stress (Hasanuzzaman and Fujita, 2013). However, application of NO caused a slight increase in accumulation of glycinebetaine in the wheat plants. In contrast to our results, however, a NO-induced decrease in proline accumulation was also observed in tobacco (Lopez-Carrion et al., 2008).

Some previous studies have shown that yield and yield components such as number of seeds, yield per plant, and 100-seed weight of wheat decrease under saline stress (Anjum et al., 2008). Our findings related to yield components have shown that the effect of salinity was more damaging to grain filling. This is in agreement with some previous studies (Shahbaz et al., 2008; Perveen et al., 2010, 2011). In our study, exogenous application of NO as a foliar spray significantly increased yield per plant, number of seeds per plant, and 100-seed weight of wheat cultivar S-24 under nonstressed conditions, while NO enhanced grain yield per plant under saline conditions.

Application of NO reduced the accumulation of Na^+ and enhanced that of K^+ (Zhang et al., 2004). Consequently, exogenous application of NO proved to be helpful for crop establishment (Zheng et al., 2009). Carden et al. (2003) reported that for salt tolerance, the Na^+/K^+ ratio is always critical, as opposed to Na^+ concentration alone. It can be said that a decreased ratio of Na^+ to K^+ avoids the salinity damage to plants under salt stress. This ratio can be decreased by the foliar application of NO (Gua et al., 2008). Ruan et al. (2004) reported that Ca^{2+} is tightly linked with the proline accumulation in wheat, and the signaling flow of NO may be cytosolic Ca^{2+}-dependent (Zhu, 2002). In our findings, exogenous application of NO decreased the accumulation of Na^+ and increased that of K^+. A negative correlation of Na^+ with yield has been found in our study (yield and Na^+, r = –0.709*). This indicates that increased Na^+ caused decreased plant yield. Lopez-Carrion et al. (2008) found that NO treatment with NaCl did not significantly affect the Cl^- concentration. In contrast, in our findings, exogenous application of varying levels of NO showed a slight decrease in shoot Cl^- under nonstressed and root Cl^- under salt stress conditions.

In conclusion, salt stress had adverse effects on the growth attributes of wheat plants. However, exogenous application of NO proved to be beneficial in enhancing plant growth parameters like dry weights and lengths of both shoot and root of salt-stressed plants. Nitric oxide application played a protective role by effectively scavenging reactive oxygen species through increased activities of antioxidant enzymes like SOD, POD, and CAT. High accumulation of free proline and total soluble proteins by exogenous application of NO regulated osmoregulation in the wheat plants under both saline and nonsaline conditions. Application of NO caused high accumulation of K^+ and low accumulation of Na^+, and it increased the yield of wheat under saline conditions.

References

Adebooye OC, Hunsche M, Noga G, Lankes C (2012). Morphology and density of trichomes and stomata of *Trichosanthes cucumerina* (Cucurbitaceae) as affected by leaf age and salinity. Turk J Bot 36: 328–335.

Akram NA, Ashraf M, Al-Qurainy F (2012). Aminolevulinic acid-induced regulation in some key physiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus* L.) under saline regimes. Sci Hortic 142: 143–148.

Anjum F, Wahid A, Javed F, Arshad M (2008). Influence of foliar applied thiourea on flag leaf gas exchange and yield parameters of bread wheat (*Triticum aestivum* L.) cultivars under salinity and heat stresses. Int J Agric Biol 10: 619–626.

Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373–399.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15.

Ashraf MA, Ashraf M, Shahbaz M (2012). Growth stage-based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. Flora 207: 388–397.

Bates LS, Waldern RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant Soil 39: 205–207.

Batool A, Ashraf M, Akram NA, Al-Qurainy F (2012). Salt-induced changes in growth, some key physio-biochemical attributes, activities of enzymatic and levels of non-enzymatic antioxidants in cauliflower (*Brassica oleracea* L.). J Hortic Sci Biotechnol 88: 231–241.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annu Rev Biochem 72: 248–254.

Carden DE, Walker DJ, Flowers TJ, Miller J (2003). Single-cell measurements of the contributions of cytosolic Na^+ and K^+ to salt tolerance. Plant Physiol 131: 676–683.

Chance M, Maehly AC (1955). Assay of catalases and peroxidases. Method Enzymol 2: 764–817.

Chen S, Gollop N, Heuer B (2009). Proteomic analysis of salt-stressed tomato (*Solanum lycopersicum*) seedlings: effect of genotype and exogenous application of glycinebetaine. J Exp Bot 60: 2005–2019.

Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ (2004). ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. J Exp Bot 55: 205–212.

Fan H, Guo S, Jiao Y, Zhang R, Li J (2007). Effects of exogenous nitric oxide on growth, active oxygen species metabolism, and photosynthetic characteristics in cucumber seedlings under NaCl stress. Front Agric China 1: 308–314.

Farooq M, Basra SMA, Wahid A, Rehman H (2009). Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). J Agron Crop Sci 14: 220–225.

Giannopolitis CN, Ries SK (1977). Superoxide dismutase in higher plants. Plant Physiol 59: 309–314.

Grieve CM, Grattan SR (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70: 303–307.

Guo Y, Tian Z, Yan D, Zhang J, Qin P (2008). Effects of nitric oxide on salt stress tolerance in *Kosteletzkya virginica*. Life Sci J 6: 67–75.

Hameed M, Nawaz T, Ashraf M, Naz N, Batool R, Ahmad MSA, Riaz A (2013). Physioanatomical adaptations in response to salt stress in *Sporobolus arabicus* (Poaceae) from the Salt Range, Pakistan. Turk J Bot 37: 715–724.

Hasanuzzaman M, Fujita M (2013). Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. Ecotoxicollogy 22: 584–596.

Hasanuzzaman M, Hossain MA, Fujita M (2011). Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnol Rep 5: 353–365.

Hasanuzzaman M, Nahar K, Alam MM, Fujita M (2012) Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. Aust J Crop Sci 6: 1314–1323.

Jin CW, Du ST, Zhang YS, Tang C, Lin XY (2009). Atmospheric nitric oxide stimulates plant growth and improves the quality of spinach (*Spinacia oleracea*). Ann Appl Biol 155: 113–120.

Joseph B, Jini D (2010). Insights into the role of antioxidative enzymes for salt tolerance in plants. Int J Bot 6: 456–464.

Kanwal H, Ashraf M, Shahbaz M (2011). Assessment of salt tolerance of some newly developed and candidate wheat (*Triticum aestivum* L.) cultivars using gas exchange and chlorophyll fluorescence attributes. Pak J Bot 43: 2693–2699.

Kausar F, Shahbaz M (2013). Interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (*Triticum aestivum* L.). Pak J Bot 45(SI): 67–73.

Lopez-Carrion AI, Castellano R, Rosales MA, Ruiz JM, Romero L (2008). Role of nitric oxide under saline stress: implications on proline metabolism. Biol Plant 52: 587–591.

Naheed G, Shahbaz M, Akram NA, Ashraf M (2008). Interactive effect of rooting medium application of phosphorus and NaCl on plant biomass and mineral nutrients of rice (*Oryza sativa* L.). Pak J Bot 40: 1601–1608.

Noreen S, Ashraf M (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. Pak J Bot 40: 1657–1663.

Perveen S, Shahbaz M, Ashraf M (2010). Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. Pak J Bot 42: 3073–3081.

Perveen S, Shahbaz M, Ashraf M (2011). Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. Pak J Bot 43: 2463–2468.

Perveen S, Shahbaz M, Ashraf M (2012a). Changes in mineral composition, uptake and use efficiency of salt stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. Pak J Bot 44: 27–35.

Perveen S, Shahbaz M, Ashraf M (2012b). Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress? J Appl Bot Food Qual 85: 41–48.

Ruan HH, Shen WB, Xu LL (2004). Nitric oxide modulates the activities of plasma membrane ATPase and PPase in wheat seedling roots and promotes the salt tolerance against salt stress. Acta Bot Sin 46: 415–422.

Saadia M, Jamil A, Akram NA, Ashraf M (2012). A study of proline metabolism in canola (*Brassica napus* L.) seedlings under salt stress. Molecules 17: 5803–5815.

Shahbaz M, Ashraf M (2013). Improving salinity tolerance in cereals. Crit Rev Plant Sci 32: 237–249.

Shahbaz M, Ashraf M, Akram NA, Hanif A, Hameed S, Joham S, Rehman R (2011). Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). Acta Physiol Plant 33: 1113–1122.

Shahbaz M, Ashraf M Al-Qurainy F, Harris PJC (2012). Salt tolerance in selected vegetable crops. Crit Rev Plant Sci 31: 303–320.

Shahbaz M, Ashraf M, Athar HR (2008). Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? Plant Growth Regul 55: 51–64.

Shahbaz M, Zia B (2011). Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions? J Appl Bot Food Qual 84: 54–60.

Shahbaz M, Noreen N, Perveen S (2013). Triacontanol modulates photosynthesis and osmoprotectants in canola (*Brassica napus* L.) under saline stress. J Plant Interac 8: 350-359.

Shaheen HL, Shahbaz M, Ullah I, Iqbal MZ (2012). Morpho-physiological responses of cotton (*Gossypium hirsutum* L.) to salt stress. Int J Agric Biol 14: 980–984.

Snedecor GR, Cochran WG (1989). Statistical Methods. 8th ed. Ames, IA, USA: Blackwell Publishing.

Tuncz-Ozdemir M, Miller G, Song L, Kim J, Sodek A, Koussevitzky S, Misra AN, Mittler R, Shintani D (2009). Thiamin confers enhanced tolerance to oxidative stress in *Arabidopsis.* Plant Physiol 151: 421–432.

Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci 163: 515–523.

Wang XY, Shen WB, Xu LL (2004). Exogenous nitric oxide alleviates osmotic stress-induced membrane lipid peroxidation in wheat seedling leaves. Physiol Mol Biol Plant 30: 195–200.

Wolf B (1982). A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun Soil Sci Plant Anal 13: 1035–1059.

Wu X, Zhu W, Zhang H, Ding H, Zhang HJ (2010). Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (Lycopersicom esculentum Mill.). Acta Physiol Plant 10: 1007–1173.

Zanardo DIL, Zanardo FML, Ferrarese MLL, Magalhaes JR, Ferrarese-Filho O (2005). Nitric oxide affecting seed germination and peroxidase activity in canola (*Brassica napus* L.). Physiol Mol Biol Plant 11: 81–86.

Zhang LP, Mehta SK, Liu ZP, Yang ZM (2008). Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant Cell Physiol 49: 411–419.

Zhang Y, Han X, Chen X, Jin H, Cui X (2009). Exogenous nitric oxide on antioxidative system and ATPase activities from tomato seedlings under copper stress. Sci Hortic 123: 217–223.

Zhang Y, Wang L, Liu Y, Zhang Q, Wei Q, Zhang W (2006). Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na^+/H^+ antiport in the tonoplast. Planta 224: 545–555.

Zhang YY, Liu J, Liu YL (2004). Nitric oxide alleviates growth inhibition of maize seedlings under salt stress. J Plant Physiol Mol Biol 30: 455–459 (in Chinese).

Zheng CF, Dong JG, Liu FL, Dai TB, Liu WC, Jing Q, Cao WX (2009). Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. Environ Exp Bot 67: 222–227.

Zhou B, Guo Z, Xing J, Huang B (2005). Nitric oxide is involved in abscisic acid-induced antioxidant activities in *Stylosanthes guianensis*. J Exp Bot 56: 3223–3228.

Zhu JK (2002). Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 5: 247–273.