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Exogenously applied nitric oxide confers tolerance to salinity-induced oxidative stress in two maize (*Zea mays* L.) cultivars differing in salinity tolerance

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Abstract: A glasshouse study was conducted to examine the ameliorative effects of exogenously applied nitric oxide (NO) on salinity-induced oxidative defense mechanisms and some vital metabolic attributes in two maize cultivars differing in salinity tolerance. It also aimed to compare the effects of two different modes of NO application on different parameters of plants grown under a saline regime. Two maize cultivars, namely DK 5783 (salt-tolerant) and Apex 836 (salt-sensitive), were subjected to saline stress, and two levels of NO were applied as presowing or foliage spray. Saline stress caused significant suppression in total fresh and dry biomass, maximum fluorescence yield (F_v/F_m), leaf water potential, and total chlorophylls ($a + b$) in the plants of both maize cultivars. On the other hand, it increased leaf osmotic pressure, proline accumulation, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations, membrane permeability, and the activities of some key antioxidant enzymes, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). Exogenously applied NO in both modes partly alleviated the adverse effects of salinity on plants of both maize cultivars. In most cases there seemed to be no difference between seed and foliar application of NO in alleviating the adverse effects of salt stress on maize plants. NO partially improved salt tolerance of maize plants; it reduced Na^+ but increased N, K^+, Ca^{2+}, and P in the maize plants under saline regimes. The NO treatment conferred enhanced tolerance to salinity by reducing MDA and H_2O_2 levels and antioxidant enzymes such as SOD, CAT, and POD, as well as enhancing photosynthetic pigments under salinity stress.

Key words: Leaf water potential, maize, nitric oxide, oxidative stress, salt tolerance

1. Introduction

Reduced plant growth and development under salinity stress are mainly due to nutrient imbalance, osmotic stress, and specific ion toxicity, which cause oxidative stress because of excess generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical (Serrato et al., 2004; Ashraf and Foolad, 2007; Ashraf, 2009; Miller et al., 2010; Habib et al., 2012; Iqbal and Ashraf, 2013). These ROS are thought to be injurious because they can significantly damage cellular metabolites/molecules, including lipids and proteins (Ashraf, 2009; Miller et al., 2010; Golldack et al., 2014; Noctor et al., 2014). However, to counteract ROS, plants upregulate their antioxidative defense mechanism by stimulating the activities of key antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Asada, 1999; Apel and Hirt, 2004; Ashraf, 2009; Sai-Kachout et al., 2013).

Induction/improvement of stress tolerance in crop plants is contemplated as a beneficial strategy to economically utilize salt-affected lands (Ashraf and Harris, 2004). Although this approach has gained considerable ground worldwide, there are still many challenges in attaining the desired outcome that need to be resolved. Alternatively, a shotgun approach has been adopted by many researchers (Ashraf and Foolad, 2005; Tanou et al., 2012; Akram and Ashraf, 2013). This approach is an immediate remedy to improve plant growth development under stress or nonstressed conditions by exogenously applied macro-/micronutrients, plant growth regulators/hormones, osmoprotectants, and antioxidants (Ashraf and Foolad, 2005, 2007; Ali et al., 2008; Akram and Ashraf, 2013). These can be applied via seed treatments (seed priming/soaking) or foliage spray, as well as through rooting media (Ashraf and Foolad, 2007).

Nitric oxide (NO) is thought to play a key role in oxidative stress responses and other related processes

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(Chen et al., 2010). For example, NO, being one of the potential growth regulators, has a protective role against various abiotic stresses such as salinity stress (Chen et al., 2010; Mihailovic and Drazic, 2011; Habib et al., 2010, 2013) and heavy metal stresses such as Cd (Besson-Bard et al., 2009), Cu (Zhang et al., 2008), and Fe (Sun et al., 2007) deficiency/toxicity. It is thought that the application of this growth regulator could attenuate the adverse effects of salt on most plants, e.g., tomato (Wu et al., 2010), wheat (Xie et al., 2008), maize (Yildiztugay et al., 2014), and rice (Habib and Ashraf, 2014). During a study with tomato plants, Wu et al. (2010) observed that rooting medium application of 100 μM NO improved plant growth under a saline regime. They attributed this growth improvement to NO-induced enhancement in photosynthetic and PS-II efficiency under saline conditions.

High salt concentration in soils or water induces ionic and osmotic stress in plants (Siddiqui et al., 2011; Khan et al., 2012; Kanwal et al., 2013; Manai et al., 2014). However, tolerant plant cells maintain ion homeostasis by vacuolar compartmentalization, extrusion of toxic ions into the external medium, and/or maintenance of a high K/Na ratio. It has been documented that exogenously applied NO significantly decreased toxic concentration of Na^+ (Bai et al., 2014; Habib and Ashraf, 2014). For example, presowing seed treatment with NO significantly decreased root and shoot sodium and chloride levels, while it enhanced potassium and calcium concentrations and K^+/Na^+ ratio in rice plants (Habib and Ashraf, 2014).

Therefore, in the present study the effects of NO applied as foliar spray or seed soaking on selected plant growth parameters such as production of ROS, activities of antioxidant enzymes, and mineral nutrition status were investigated in two genetically different maize cultivars subjected to saline stress. It was also specifically aimed to compare the mode of NO application to some key parameters measured in maize plants grown under saline conditions.

2. Materials and methods

2.1. Plant materials and treatments

A complete block design experiment with 3 replicates was planned at the Research Station of Harran University, Turkey, during May and June 2013. Two maize cultivars, namely Apex 836 and DK 5783, were selected for experimentation. Ten kilograms of air-dried loamy-clay soil was added to each plastic pot. The texture of the soil used was loamy clay; pH (1:2.5 water, v:v) was 7.3, electrical conductivity (EC) was 0.45 dS/m, K = 1.40 g/kg, and N = 1.25 g/kg. Nitrogen, P, and K were mixed into the soil at a rate of 100, 50, and 120 mg/kg as granular urea, triple superphosphate, and potassium sulfate, respectively.

After germination, three seedlings of uniform size were planted in each pot. All pots were then transferred to a glasshouse after maintaining a mean temperature and relative humidity of 27 ± 2 °C and 60%–70%, respectively. Plants were allowed to establish for 7 days before the initiation of salt treatment. In addition to the control [(c), 0 mM NaCl], salt treatment [(s), 100 mM NaCl; 5.85 g NaCl kg^{-1} soil] was employed with irrigation water. The EC of the soil was checked regularly until the completion of the experiment. Two levels of nitric oxide (3 and 6 mg L^{-1}) were applied as seed soaking (s) and foliar (f) applications. Sodium nitroprusside was used as a source of NO. For seed soaking treatment, seeds were dipped in the respective concentrations for 1 day before sowing. The first foliar spray of NO (50 mL/pot) concentrations was applied after 10 days of seed germination and was continued once a week until day 35 of the experiment. Afterwards, one plant per replicate was uprooted and washed with distilled water to remove soil particles. After measuring fresh weights, plants were dried in an oven up to constant weight and their dry weight was recorded. The remaining two plants per replicate were used for the determination of the following attributes.

2.2. Chlorophyll contents

One gram of leaf sample was ground in acetone (90%) and filtered. The filtrate was run on a UV-visible spectrophotometer (Shimadzu UV-1201, Japan) for determination of the absorbance of each sample according to Strain and Svec (1966).

2.3. Chlorophyll fluorescence

Different leaf chlorophyll fluorescence attributes were determined using leaves that had been previously dark- and light-adapted with a portable chlorophyll fluorometer (Photosynthesis Yield Analyzer Mini-PAM, Walz, Germany).

2.4. Free proline content

Fresh leaf tissues (500 mg) were ground in 10 mL of sulfosalicylic acid (3%). The supernatant (2 mL) was added to acid-ninhydrin (2 mL) and glacial acetic acid (2 mL) following the protocol proposed by Bates et al. (1973). The mixture was then kept at 100 °C in a water bath for 1 h. After cooling the samples, 4 mL of toluene was added to each sample. The samples were then vortexed, and optical density of the colored upper layer of each sample was read at 520 nm.

2.5. Leaf osmolality

Leaves were collected in a container containing liquid nitrogen and were stored at −80 °C. After 1 week, the samples were extracted and the sap was collected using a syringe. The osmolality of the sap was then determined with an osmometer (Cryo-Osmomat 030, Ganotec, Canada).

2.6. Leaf water potential

A third leaf from the top was detached from each plant before sunshine and inserted in a pressure chamber (PMS model 600, USA) for determining water potential.

2.7. Electrolyte leakage

Fresh leaf tissues (0.2 g) were cut into small pieces (5 mm) and put into test tubes, each containing 10 mL of dH_2O. All test tubes were incubated at 32 °C for 2 h using a water bath according to Dionisio-Sese and Tobita (1998). The test tubes were then cooled and the electrical conductivity (EC_1) of the aliquot was determined. Afterwards, the samples were autoclaved at 121 °C for 20 min and the temperature of the samples was decreased to 25 °C, and then EC_2 measured. The following formula was used for estimating electrolyte leakage (EL): $EL = EC_1/EC_2 \times 100$.

2.8. Antioxidant enzyme assays

Fresh leaf tissues (500 mg) were extracted in Na-P buffer (50 mM; pH 7.0) containing 1% soluble polyvinylpyrrolidone. All samples were then centrifuged at 20,000 × *g* for 12 min. The supernatant was collected in autoclaved plastic vials and stored at –20 °C to determine the activities of CAT, POD, and SOD enzymes. The activity of CAT was appraised following Kraus and Fletcher (1994) by determining the consumption of hydrogen peroxide (H_2O_2) at 405 nm. The SOD activity was appraised following Beauchamp and Fridovich (1971) and was based on the ability of the enzyme to suppress the photochemical reduction of NBT. The activity of POD was measured following Chance and Maehly (1955) by adding 100 µL of the tissue extract to 3 mL of assay solution, which contained 13 mM guaiacol, 5 mM H_2O_2, and 50 mM Na-P buffer (pH 6.5). The POD activity was appraised as the change in absorbance at 470 nm min^{-1} mg^{-1} of protein. The Bradford (1976) protocol was used to quantify the total soluble protein contents.

2.9. Lipid peroxidation

The protocol described by Weisany et al. (2012) was employed with some modifications to measure lipid peroxidation in the leaf samples by measuring malondialdehyde (MDA) content.

2.10. Hydrogen peroxide contents

H_2O_2 in the leaf samples was quantified following Loreto and Velikova (2001). A fresh leaf sample (500 mg) was ground in 3 mL of trichloroacetic acid (1%; w/v) and then the extract was centrifuged at 10,000 × *g* at 4 °C for 10 min. An aliquot (0.75 mL) was mixed with 0.75 mL of K-phosphate buffer (10 mM; pH 7.0) and 1.5 mL of KI (1 M). The absorbance of the mixture was determined at 390 nm and H_2O_2 contents were expressed as µmol g^{-1} FW.

2.11. Nutrient analysis

The analysis of inorganic nutrients was conducted using dry plant samples. Total N was determined using the Kjeldahl method**.** For the analysis of other nutrients, the dried and ground samples were ashed in a muffle furnace at 550 °C for 6 h. The white ash was dissolved in 5 mL of hot HCl (2 M) and the final volume was made to 50 mL with dH_2O. Na, K, and Ca were analyzed according to Chapman and Pratt (1982), while phosphorus was analyzed with the vanadate-molybdate method according to Jackson (1962).

2.12. Statistical analysis

Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed using the SAS GLM procedure to examine differences between the two cultivars and treatments at $P \leq 0.05$.

3. Results

3.1. Key growth parameters

Shoot fresh and dry weights of both cultivars decreased significantly under saline stress; however, Apex 836 was more adversely affected by salinity stress compared to DK 5783 (Table 1). Relative changes (percentage of control) of Apex 836 and DK 5783 grown at salinity were 49.2% and 58.7% for fresh weight and 55.1% and 60.9% for dry weight, respectively (Table 1). Both modes of exogenous application of nitric oxide improved biomass production (fresh and dry weights) in both maize cultivars. Although there were no apparent significant differences between the effects of both modes of NO in most cases, exogenously applied NO as a foliar spray was found to be more effective than the seed treatment, particularly for Apex 836 (Table 1). MANOVA of the data showed marked differences between the two cultivars and different treatments in terms of fresh and dry weights (Table 1).

Both maximum fluorescence yield (F_v/F_m) and total chlorophyll content decreased significantly due to salinity stress. In contrast, membrane permeability increased in both cultivars. DK 5783 was less affected than Apex 836 with respect to these data. Exogenously applied NO as seed or foliar applications improved all three key parameters. Overall, there seemed to be no significant differences between the effects of both modes of NO applications in both cultivars in most cases (Table 2). The MANOVA results show that there were significant differences between the cultivars and treatments for F_v/F_m and membrane stability (MS), but not for total chlorophyll content, at $P \leq 0.05$ (Table 2).

Leaf water potential decreased, although leaf osmolality (LO) and proline (Pro) content increased markedly by salinity stress in both cultivars. Leaf water potential of the salt-sensitive cultivar, Apex 836, was more adversely affected by salinity stress. Furthermore, salinity stress caused a higher increase in leaf osmolality in the salt-sensitive cultivar (Table 3). MANOVA revealed significant differences between the cultivars and treatments in terms of leaf water potential (Ψl), LO, and Pro (Table 3).

Table 1. Fresh and dry weights of different cultivars of maize grown in salt with or without different levels of nitric oxide (mg/L) applied as different modes.

Cultivars	Treatments	FW (g)	RC	DW (g)	RC
DK 5783	C	16.7 a	100.0	1.84 a	100
	S	9.8 c	58.7	1.12 d	60.9
	sNO 3	11.2 b	67.1	1.20 c	65.2
	sNO 6	12.4 b	74.3	1.28 b	69.6
	fNO 3	12.3 b	73.7	1.26 bc	68.5
	fNO 6	12.1 b	72.5	1.21 bc	65.7
Apex 836	C	12.8 a	100.0	1.27 a	100
	S	6.3 c	49.2	0.70 d	55.1
	sNO 3	6.9 c	53.9	0.76 c	59.9
	sNO 6	7.1 bc	55.5	0.78 bc	61.4
	fNO 3	7.5 b	58.6	0.80 bc	63.0
	fNO 6	7. 6b	59.4	0.82 b	64.6
Cultivars × treatments		*	*	*	*

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$. RC: Relative changes compared to the controls.

Table 2. Maximum fluorescence yield (F_v/F_m), membrane stability (MS), and total chlorophyll (mg kg^{-1} FW) of different cultivars of maize grown in salt with or without different levels of nitric oxide (mg/L) applied as different modes.

Cultivars	Treatments	Fv/FM	MS (%)	Chl.
DK 5783	C	0.63 a	17 c	1266 a
	S	0.57 c	25 a	1054 d
	sNO 3	0.60 b	22 b	1125 c
	sNO 6	0.59 b	21 b	1126 c
	fNO 3	0.60 b	20 b	1185 b
	fNO 6	0.59 b	21 b	1165 b
Apex 836	C	0.63 a	18 d	1196 a
	S	0.55 c	29 a	1011d
	sNO 3	0.55 c	24 c	1072 b
	sNO 6	0.56 bc	25 bc	1072 b
	fNO3	0.57 b	25 bc	1055 c
	fNO 6	0.56 bc	26 b	1045 c
Cultivars × treatments		*	*	ns

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$; ns: not significant.

Both modes of exogenous NO applications improved Ψl and greatly suppressed Pro and LO. Foliar application of NO at 3 mg L^{-1} was found to be more effective in elevating Ψl in both cultivars. However, seed application of NO at 3 and 6 mg L^{-1} was more effective in terms of reducing LO and Pro in both cultivars in most cases.

3.2. Mineral nutrients

Salinity stress caused a significant rise in sodium (Na^+) in the leaves of both maize cultivars. Apex 836 accumulated significantly higher Na^+ in its leaves than the salt-tolerant cultivar DK 5783 (Table 4). Both modes of NO application reduced Na^+ content to levels still much higher than that in the control plants of both cultivars. In contrast, concentrations of essential nutrients such as N, P, Ca^{2+}, and K^+ in the leaves of both cultivars were lowered by salinity stress. However, the levels of these nutrients were lower in the salt-sensitive cultivar Apex 836 than in the salt-tolerant cultivar (Tables 4 and 5). There were significant differences between the cultivars and treatments for all the nutrients tested by MANOVA at $P \leq 0.05$ (Tables 4 and 5).

Exogenously applied NO as a seed presowing treatment or foliar application caused marked reduction in leaf Na^+ accumulation, yet resulted in an increase in the other analyzed elements. Seed applications of NO at both doses (3 and 6 mg L^{-1}) were more effective in lowering leaf Na^+ in both cultivars. The higher dose (6 mg L^{-1}) of NO as a seed or foliar treatment seemed to be more effective in elevating the levels of other leaf nutrients in most cases.

3.3. Antioxidant enzymes and reactive oxygen species

Salinity of the root-growing medium caused a marked rise in the activities of POX, CAT, and SOD in both maize cultivars. The activities of these enzymes were higher in salt-tolerant DK 5783 than in salt-sensitive Apex 836. NO applied exogenously through both modes caused a marked suppression in the activities of all analyzed antioxidant enzymes; however, there seemed to be no consistent pattern of the effects of NO doses or modes on regulating the activities of the antioxidant enzymes. Although significant differences between the cultivars and treatments were obtained for SOD and CAT, there were no significant differences between the cultivars and treatments for POX activity according to MANOVA at $P \leq 0.05$ (Table 6).

Salinity stress caused higher accumulation of MDA and H_2O_2 in the leaves of both cultivars, and this accumulation was higher in the salt-sensitive cultivar grown under saline stress (Table 7). However, both modes of NO application effectively lowered leaf MDA and H_2O_2 contents in both maize cultivars. Exogenously applied NO as seed

Table 3. Leaf water potential (Ψl, MPa), leaf osmolality (LO, osmol/kg), and proline (Pro, μmol/g) of different cultivars of maize grown in salt with or without different levels of nitric oxide (mg/L) applied as different modes.

Cultivars	Treatments	**Ψl**	LO	Pro
DK 5783	C	–0.33 a	0.045 d	1.09 d
	S	–1.44 d	0.129 a	2.89 a
	sNO 3	–1.29 bc	0.102 c	2.32 c
	sNO 6	–1.32 c	0.103 c	2.35 c
	fNO 3	–1.24 b	0.114 b	2.45 b
	fNO 6	–1.32 c	0.112 b	2.39 bc
Apex 836	C	–0.31 a	0.041 d	1.12 d
	S	–1.62 d	0.138 a	2.62 a
	sNO 3	–1.36 c	0.112 c	2.36 c
	sNO 6	–1.38 c	0.125 b	2.35 c
	fNO 3	–1.25 b	0.122 b	2.42 b
	fNO 6	–1.39 c	0.122 b	2.44 b
Cultivars × treatments		*	*	*

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$.

Table 4. Sodium and nitrogen concentrations (mmol/kg) of different cultivars of maize grown in salt with or without different levels of nitric oxide (mg/L) applied as different modes.

Cultivars	Treatments	Na	N
DK 5783	C	36 d	1157 a
	S	327 a	886 d
	sNO 3	255 c	955 c
	sNO 6	265 c	985 b
	fNO 3	278 b	985 b
	fNO 6	278 b	995 b
Apex 836	C	30 d	1126 a
	S	397 a	841 f
	sNO 3	335 c	942 c
	sNO 6	336 c	962 b
	fNO 3	349 b	896 e
	fNO 6	354 b	925 d
Cultivars × treatments		*	*

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$.

Table 5. Phosphorus, calcium, and potassium concentrations (mM/kg) of different cultivars of maize grown in salt with or without different levels of nitric oxide (mg/L) applied as different modes.

Cultivars	Treatments	P	Ca	K
DK 5783	C	67 a	175 a	354 a
	S	33 d	114 d	258 d
	sNO 3	38 c	125 c	278 b
	sNO 6	42 b	129 bc	265 c
	fNO 3	42 b	135 b	269 c
	fNO 6	44 b	129 bc	278 b
Apex 836	C	61 a	162 a	342 a
	S	27 d	93 c	222 d
	sNO 3	36 c	124 b	271 b
	sNO 6	35 c	127 b	261 bc
	fNO 3	41 b	126 b	265 bc
	fNO 6	40 b	124 b	256 c
Cultivars × treatments		*	*	*

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$.

Table 6. Superoxide dismutase (SOD: unit mg protein^{-1} min^{-1}), catalase (CAT: unit × 100/mg protein), and peroxidase (POX: ΔA_{470} min^{-1} mg protein^{-1}) of different cultivars of maize grown in salt with or without different levels of diurea (mg/L) applied as different modes.

Cultivars	Treatments	SOD	CAT	POX
DK 5783	C	48 d	1.33 d	8.19 c
	S	172 a	2.93 a	36.16 a
	sNO 3	127 b	2.23 bc	22.89 b
	sNO 6	125 bc	2.34 b	23.98 b
	fNO 3	117 c	2.14 c	24.98 b
	fNO 6	132 b	2.25 bc	23.12 b
Apex 836	C	47 d	1.33 c	8.97 c
	S	158 a	2.67 a	35.28 a
	sNO 3	105 c	2.05 b	22.35 b
	sNO 6	109 c	2.12 b	23.33 b
	fNO 3	102 c	2.02 b	25.23 b
	fNO 6	124 b	2.05 b	22.36 b
Cultivars × treatments		*	*	ns

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at $P \leq 0.05$. MANOVA: *$P \leq 0.05$.

treatment at 6 mg L^{-1} and foliar application of NO at 3 mg L^{-1} was more effective in lowering the ROS tested in the present study. Based on MANOVA, marked differences were observed among the cultivars and treatments for both MDA and H_2O_2 (Table 7).

4. Discussion

It is thought that seed treatments and foliar applications of a variety of plant growth substances to plants under stressful environments may have differential promotive effects on most crops (Khan et al., 2006; Athar et al., 2009; Plaut et al., 2013). Therefore, the present investigation aimed to assess the influence of different modes of NO application, as seed soaking or foliar application, at the vegetative growth stage of two maize cultivars differing in salinity tolerance. Both modes of NO at 3 or 6 mg L^{-1} enhanced plant growth of both maize cultivars. Although no significant differences were found among the effects of both modes of NO on plant growth of maize grown under salt stress in most cases, exogenously applied NO as a foliar spray seemed to be more effective in mitigating the adverse effects of salt stress than the seed treatment of NO. NO-enhanced stress tolerance and improved yield have been observed in different crops such as wheat (Zheng et al., 2009; Kausar and Shahbaz, 2013) and rice (Habib et al., 2010; Habib and Ashraf, 2014). Improvement in salinity tolerance in various plants/crops has been shown to be linked with increased growth and yield as well as multiple metabolic adaptations (Batool et al., 2014; Gupta and Huang, 2014), including low accumulation of ROS (Fayez and Bazaid, 2014), low uptake of toxic ions (Kader and Lindberg, 2005; Sabir and Ashraf, 2007), high accumulation of vital osmoprotectants (Ashraf and Foolad, 2007; Kaya et al., 2013), upregulation of the oxidative defense system (Ashraf, 2009; Akram et al., 2012), and efficient photorespiratory machinery (Muranaka et al., 2002). Therefore, in the present study, NO-induced high biomass production may be ascribed to low uptake of Na^+ as well as low accumulation of ROS. This NO-induced increase in biomass production in maize plants may have been due to regulation of cellular osmotic adjustment, a key physiological process of maintaining cell turgidity (Ke et al., 2013; Habib and Ashraf, 2014). For example, exogenous application of NO significantly improved the leaf water potential (Ψ_w) of maize plants and was found to have a positive relationship with increased plant biomass production.

In the present study, salinity stress caused a marked decline in the maximum fluorescence yield (F_v/F_m) of both

Table 7. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations in leaves of different cultivars of maize grown in salt with or without different levels of diurea (mg/L) applied as different modes.

Cultivars	Treatments	H_2O_2 (μmol g^{-1} DW)	MDA (nmol g^{-1} FW)
DK 5783	C	1.17 d	1.34 d
	S	6.54 a	10.27 a
	sNO 3	4.31 b	7.26 b
	sNO 6	3.81 c	6.79 b
	fNO 3	3.72 c	6.82 b
	fNO 6	4.55 b	7.80 b
Apex 836	C	1.23 d	1.56 d
	S	8.66 a	13.23 a
	sNO 3	6.26 b	9.56 b
	sNO 6	5.27 c	8.64 c
	fNO 3	5.49 c	8.25 c
	fNO 6	6.33 b	9.86 b
Cultivars × treatments		*	*

NO: Nitric oxide; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg/L). Means marked with different letters in the same column within the same cultivar indicate significant difference between treatments at P ≤ 0.05. MANOVA: *P ≤ 0.05.

maize cultivars, and the reduction was higher in the salt-sensitive maize cultivar Apex 836 than that in salt-tolerant DK 5783. The value of F_v/F_m is often used as an indicator of stress tolerance or photoinhibition in PS-II activity (Calatayud and Barreno, 2004). Several recent studies have reported that saline stress can result in alterations of leaf fluorescence in a broad range of crops such as sunflower (Akram et al., 2009), okra (Saleem et al., 2011), wheat (Habib et al., 2013; Perveen et al., 2013), and eggplant (Shaheen et al., 2012). This reduction in maximum fluorescence yield (F_v/F_m) by salinity stress might be due to the inactivation and destruction of the PS-II reaction center (Santos et al., 2001; Yan et al., 2012; Ashraf and Harris, 2013; Dong et al., 2014). In the present study, PS-II activity (F_v/F_m) of both maize cultivars increased due to exogenously applied NO in salt-stressed maize plants, which is analogous to the findings of Kausar and Shahbaz (2013), who documented improved chlorophyll fluorescence in salt-stressed wheat plants due to exogenously applied NO. However, contradictory evidence was reported by Yang et al. (2001), who suggested that foliar application of NO alone reduced the activity of photosystem II. However, the gap between NO-induced changes in the photosynthesis linked to either PS-I or PS-II still needs to be elucidated.

Increased plant growth is linked to leaf photosynthetic rate, ultimately depending on quantity of leaf photosynthetic pigments such as total chlorophyll. Salt stress is thought to cause degradation of leaf chlorophyll contents, which results in reduced plant photosynthetic rate and ultimately reduced biomass production. Several studies have shown that exogenously applied organic compounds mitigated the adverse effects of salt stress on leaf photosynthetic pigments, thereby resulting in enhanced biomass production (Ali et al., 2007; Nawaz and Ashraf, 2010; Ali and Ashraf, 2011). In the present study, exogenously applied NO was effective in enhancing the leaf chlorophyll of maize plants and was positively linked to higher photosynthetic rates and hence higher biomass production. It has also been reported that NO could enhance photosynthetic pigment contents in wheat plants grown under salt regimes (Ruan et al., 2002). The enhanced photosynthetic pigment contents by exogenous NO were linked to lowered lipid peroxidation and ROS production in plants (Hung et al., 2002). In the present study, it was also observed that exogenously applied NO lowered MDA and H_2O_2 contents in the leaves of salt-treated maize cultivars (Table 7). These findings clearly suggest that NO plays a vital role in mitigating salt-induced oxidative damage in

maize plants.

One of the key components of plant salt tolerance is the ion homeostasis mechanism. Salt stress causes the impairment of this mechanism by accumulating toxic ions such as Na^+ and Cl^- in various parts of the plant (Munns and Tester, 2008). In this study, salinity reduced mineral nutrients such as N, P, K^+, and Ca^{2+}, but enhanced Na^+ accumulation in the leaves of salt-stressed maize plants. However, exogenously applied NO as seed or foliar treatment lowered Na^+ contents, yet enhanced N, P, K^+, and Ca^{2+} contents in both maize cultivars under saline conditions, which is consistent with several earlier reports (Khan et al., 2012; Bai et al., 2014; Habib and Ashraf, 2014). Similar results were reported by Habib and Ashraf (2014), who observed that presowing treatment of rice seed with NO significantly decreased Na^+ and Cl^-, while it improved Ca^{2+} and K^+ contents in both shoots and roots of salt-stressed rice plants. Furthermore, it has also been reported that foliar-applied NO (0.09 mM) improved seedling growth of cotton; moreover, it has been suggested that this growth enhancement may have occurred due to low uptake of Na^+ and enhanced accumulation of some essential nutrients such as Ca^{2+} and K^+ (Liu et al., 2014). Therefore, the results of the present study indicate that exogenously applied NO has an effective role in cellular ion homeostasis, causing enhanced uptake of N, P, K^+, and Ca^{2+}. Consequently, we can suggest that NO may be involved in improving the uptake of essential nutrients, resulting in higher biomass of maize plants.

Salinity stress causes oxidative stress by producing high concentrations of ROS in plants, resulting in perturbation of different physiobiochemical processes (Ashraf, 2009; Gupta and Huang, 2014). As one of the defensive mechanisms under stress conditions, antioxidant enzymes such as POD, SOD, and CAT play crucial roles in counteracting ROS (Wang et al., 2008; Akram et al., 2012; Perveen et al., 2013). In the present investigation, it has also been observed that saline stress caused increased activities of CAT, SOD, and POX in both maize cultivars, and they were relatively better in high biomass-producing maize cultivar DK 5783 as compared to those in the salt-sensitive cultivar Apex 836. However, exogenously applied NO lowered the activities of all the examined antioxidant enzymes and the levels of H_2O_2 and MDA in the maize plants exposed to saline stress (Tables 6 and 7). Ashraf and Akram (2009) suggested that plants with greater antioxidant potential are better able to scavenge these ROS and hence have greater stress tolerance. In view of these results, it is suggested that NO application reduced oxidative damages to membranes of cellular organelles, which is evident by the lower level of H_2O_2 and MDA in both maize cultivars. In the present study, activities of antioxidant enzymes (SOD, POD, and CAT) increased under saline regime followed by exogenous application of NO via different modes. Recently, Khan et al. (2012) observed that exogenously applied NO (0.2 mM) alleviated NaCl-induced adverse effects on the growth of mustard plants by improving the activities of SOD, CAT, POX, APX, and GR antioxidative enzymes. They attributed this salinity tolerance in mustard plants to NO-induced improvement in antioxidative defense systems and ion homeostasis. In addition, Manai et al. (2014) suggested that rooting medium application of NO mitigates salt-induced oxidative damage in tomato plants. They showed that exogenous application of NO (100 and 300 μM) markedly enhanced activities of SOD, APX, GR, and POD enzymes and raised the levels/activities of ascorbate, nitrite reductase, and nitrate reductase under saline conditions in tomato plants.

It can be concluded that exogenously applied NO improved water and ion homeostasis as well as redox balance, which led to enhanced photosynthetic capacity and growth of both maize cultivars. The cultivars used differed in salinity tolerance, DK 5783 being salt-tolerant and Apex 836 salt-sensitive. The growth-promoting effect of NO application in maize plants also depends on their genotypic potential. Moreover, exogenous application of NO in improving salt tolerance in crops is an economically viable strategy.

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