EFFECT OF EXOGENOUSLY APPLIED NITRIC OXIDE ON WATER RELATIONS AND IONIC COMPOSITION OF RICE (ORYZA SATIVA L.) PLANTS UNDER SALT STRESS

NOMAN HABIB1 AND MUHAMMAD ASHRAF2,3*

1Department of Botany, University of Agriculture, Faisalabad-Pakistan
2University College of Agriculture, University of Sargodha, Sargodha
3Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia
*Corresponding author’s e-mail: ashrafbot@yahoo.com

Abstract

The aim of present experiment was to assess the effect of exogenously applied nitric oxide on water relation attributes and pattern of uptake of inorganic ions in rice plants under salt stress. The experiment comprised four rice cultivars, two coarse (KS-282 and IRRI-6) and two fine (Shaheen Basmati and Basmati PB-95) rice cultivars, two NaCl levels (0 and 80 mM), and three levels (0, 0.1, and 0.2 mM) of nitric oxide. Salt stress caused a significant increase in leaf water and osmotic potentials while decreased leaf turgor potential and relative water content in all four rice cultivars. Of inorganic ions, shoot and root Cl- and Na+ concentrations increased significantly, while in contrast, K+ and Ca2+ concentrations and K+/Na+ ratio decreased markedly. Pre-sowing seed treatment with nitric oxide significantly decreased leaf osmotic and water potentials and shoot and root Cl- and Na+ concentrations, while it increased leaf relative water content, leaf turgor potential, K+ and Ca2+ concentrations and K+/Na+ ratio in both shoots and roots of salt stressed rice plants. Of nitric oxide levels, 0.1 mM was more effective, while of the four rice cultivars, Shaheen basmati and IRRI-6 performed better as compared to the other two cultivars.

Introduction

Salinity-induced reduction in growth and development occurs primarily due to osmotic stress, nutrient imbalance and specific ion toxicity which result in altered hormonal level and oxidative damage due to over-production of reactive oxygen species (ROS) (Ashraf & Foolad, 2007; Ashraf, 2009; Habib et al., 2012; Iqbal & Ashraf, 2013). Apart from salt stress, plants also face osmotic stress when subjected to saline environment because accumulation of soluble salts decrease the water potential of saline soils which results in limited water availability in plants and disturbance of cellular ion balance which further cause ion toxicity (Ashraf & Harris, 2004). Both osmotic and ionic effects are known to hamper plant growth (Apes & Blumwald, 2002; Munns et al., 2006; Ashraf et al., 2010). As higher Cl- and Na+ contents are toxic for plants because they not only decrease the synthesis of chlorophyll but further results in impairment of chlorophyll fluorescence and gas exchange attributes (Ashraf, 2004; Moradi & Ismail, 2007; Ashraf & Ashraf, 2012; Habib et al., 2013).

Nitric oxide (NO) is a redox-active gaseous molecule which is lipophilic in nature (Krasylenko et al., 2010; Lin et al., 2012). Nitric oxide acts as an important regulatory molecule at different stages of plant development starting from germination (Liborius et al., 2006; Bethke et al., 2007; Habib et al., 2010), to regulation of various physiological processes in crop plants (Habib et al., 2013). Besides its role as a growth regulator, nitric oxide also has a protective role against different abiotic stresses including salt stress (Zhao et al., 2007; Habib et al., 2010, 2013) and drought stress (Gracia-Mata & Lamattina, 2001). Exogenous application of nitric oxide through different modes has been reported to be effective in improving salt tolerance by increasing proton pump activity in the seedlings of maize (Zhang et al., 2006) and enhancing seed germination rate and the activity of antioxidant enzymes in wheat (Zheng et al., 2009). Of various modes of exogenous application, pre-sowing seed treatment with NO is believed to be effective in alleviating the salt-induced adverse effects in different crops including rice (Habib et al., 2010; 2013).

Rice plant is highly sensitive to salt stress especially at the seedling and reproductive stages (Pearson & Ayers, 1960; Zeng et al., 2003). Salt-induced adverse effects appear in rice within 24 h of NaCl treatment such as ionic effect which is resulted due to excessive Na+ and Cl- accumulation in leaf tissues (Rosamel & Flowers, 2009). Salt stress causes a marked reduction in seed germination and fresh and dry weights of rice seedlings (Habib et al., 2010). It also decreases chlorophyll content and gas exchange attributes and alters various chlorophyll fluorescence attributes in rice (Moradi & Ismail, 2007; Habib et al., 2013). As nitric oxide has a protective role against salt-induced damages in crop plants, so our main objective of the present study was to assess the effect of exogenously applied nitric oxide as pre-sowing seed treatment on some vital physiological parameters of salt stressed rice plants.

Materials and Methods

The experiment was carried out in a net-house of the Botanical Garden of University of Agriculture, Faisalabad, under natural conditions (daylength 13.8 h; PPFD, 1275 μmol m-2 s-1; relative humidity 45.2%; day and night temperatures 36±3°C and 27±2°C, respectively). Seeds of four rice cultivars were used in this experiment. Of these four rice cultivars, seeds of two coarse rice cultivars (KS-282 and IRRI-6) were obtained from the Rice Research Institute, Kala Shah Kaku, while those of fine rice cultivars (Basmati PB-95 and Shaheen Basmati) from the Soil Salinity Research Institute, Pindi Bhattian. The seeds were surface sterilized for 5 min with sodium hypochlorite solution (10%) and then washed with sterilized distilled water three times. After sterilization, seeds were soaked in three different levels (0, 0.1, and 0.2
mM) of sodium nitroprusside (a nitric oxide donor) for 20 h. Nursery of rice seedlings was grown in small plastic tubes (45×66×23 cm) and thirty-day-old seedlings were transplanted in other plastic tubes (each filled with 16 kg soil) arranged in a completely randomized design with four replicates. One week after transplantation when these seedlings were established the NaCl treatment (0 and 80 mM of NaCl) was applied by a gradual increase. Data for water relation attributes and inorganic ion contents were recorded 15 days after the start of NaCl treatment.

**Leaf water potential:** For estimating water potential ($\Psi_w$) a fully grown top second leaf was removed at the vegetative stage and water potential estimated using a Scholander type pressure chamber (between 6 a.m. to 8.30 a.m.). The leaf samples were frozen in a freezer at -20°C to determine $\Psi_s$ from it after seven days.

$$\text{Relative water content of leaf (\%)} = \frac{\text{Leaf fresh weight} - \text{Leaf dry weight}}{\text{Turgid weight of leaf - dry weight}} \times 100$$

**Determining the content of mineral nutrients**

**Digestion mixture:** A chemical mixture was prepared by adding 0.42 g Se and 14 g LiSO$_4$·2H$_2$O in H$_2$O$_2$ (350 ml). Then 420 ml of H$_2$SO$_4$ were added gradually to this mixture by placing the apparatus in an ice bath. This digestion mixture was stored at 2ºC and used for the digestion of dried plant material.

**Determination of cations (K$^+$, Na$^+$, and Ca$^{2+}$):** Dried and ground plant material (0.1 g) was taken in digestion tubes and 1.5 ml of digestion mixture was added to each tube and the tubes were incubated at room temperature overnight. The tubes were placed on a hot plate and heated for 30 min while 0.1 ml of perchloric acid was poured in the tubes slowly along the side walls of digestion tubes and the process was repeated until colorless digested material obtained. The digested material from each tube was transferred in a volumetric flask and 50 ml volume was maintained. After filtration of this digested mixture, the concentration of Na$^+$, K$^+$ and Ca$^{2+}$ was determined using a Jenway PFP-7 flame spectrophotometer.

**Determination of Cl$^-$:** For determining Cl$^-$ concentration, 0.1 g dried and ground plant material was taken in digestion tubes and 10 ml of distilled water added to each tube and placed all tubes for incubation at room temperature overnight. Then the mixture was continuously heated at 80°C until the volume reduced to half, then the digestion mixture was cooled and added distilled H$_2$O to restore the original volume to 10 ml. Cl$^-$ concentration in the mixture was determined using a chloride analyzer (Model No. 926; Sherwood Scientific Ltd., Cambridge, UK).

**Statistical analysis:** A completely randomized design (CRD) arrangement with four replicates was used in this experiment and a computer software CoSTAT V 6.3 (developed by, Cohort software, Berkeley, California) was used for analyzing the data.

**Leaf osmotic potential:** The same leaf was used for determining osmotic potential using an osmometer (Wescor 5500).

**Leaf turgor potential:** Leaf turgor potential ($\Psi_p$) was determined following a method described by Nobel (1991) using the following equation:

$$\Psi_p = \Psi_w - \Psi_s$$

**Relative leaf water content:** The weight of fully grown second leaf excised from each replicate was recorded to measure relative water content (RWC). The leaf sample was immersed in distilled H$_2$O for 8 h to allow leaf to absorb excessive amount of water on their surface, then the weight of each leaf was again measured. Then the dry weight of each of these leaves was measured after drying them at 70°C in an oven and RWC determined using the following equation:

**Results**

Leaf water ($\Psi_w$) and osmotic potential ($\Psi_s$) decreased significantly due to imposition of salt stress in all four rice cultivars (Fig. 1). However, the cultivars remained indifferent with respect to $\Psi_w$ and $\Psi_s$. Nitric oxide treatment as pre-sowing seed treatment in two varying levels of sodium nitroprusside (a nitric oxide donor) decreased the values of water and osmotic potentials under both control and salt stressed conditions in all four rice cultivars. Of nitric oxide levels, 0.1 mM was more effective. Imposition of salt stress significantly decreased the leaf turgor potential ($\Psi_p$) and relative water content (RWC) in all four rice cultivars (Fig. 1). However, the values of leaf turgor potential and relative water content increased significantly due to pre-sowing seed treatment with nitric oxide under both salt stressed and non-stressed conditions. Of rice cultivars, IRRI-6 and Shaheen basmati showed comparatively more increase in the values of relative water content and leaf turgor potential at 0.1 mM level of nitric oxide.

Na$^+$ and Cl$^-$ concentrations in both shoots and roots increased significantly due to addition of NaCl salt to the root zone of all four rice cultivars (Fig. 1). Nitric oxide treatment helped significantly in decreasing the excessive uptake of Na$^+$ and Cl$^-$ in both roots and shoots of all four rice cultivars under saline conditions. Of nitric oxide levels, 0.1 mM was more effective in reducing the uptake of Na$^+$ and Cl$^-$, however, both nitric oxide levels were equally effective in reducing Cl$^-$ ion content in Basmati PB-95 under saline conditions. Both nitric oxide levels showed a non-significant effect on Na$^+$ and Cl$^-$ concentrations in both roots and shoots of all four rice cultivars under non-stressed conditions. Of rice cultivars, IRRI-6 and Shaheen Basmati showed relatively less increase in both Cl$^-$ and Na$^+$ under saline conditions.
Fig. 1. Water relation attributes and shoot and root Na⁺ and Cl⁻ in salt stressed and non-stressed plants of four rice (*Oryza sativa* L.) cultivars when different levels of nitric oxide (NO) were applied as pre-sowing seed treatment.

0, 0.1 and 0.2 mM = Levels of nitric oxide applied exogenously
Potassium (K') and calcium (Ca²⁺) contents and K'/Na⁺ ratio were decreased significantly in both roots and shoots of all four rice cultivars under saline conditions (Fig. 2). Nitric oxide treatment effectively helped in improving the K' and Ca²⁺ contents and K'/Na⁺ ratio in both shoots and roots of all four rice cultivars under salt stressed conditions. Of nitric oxide levels, 0.1 mM was more effective in improving the contents of the two ions under salt stress. While of rice cultivars, IRRI-6 and Shaheen basmati showed less decrease in root and shoot K' content under salt stressed conditions, however, all cultivars remained indifferent with respect to Ca²⁺ concentration.

Discussion

Altered functioning of various physiological processes due to NaCl-induced osmotic effect could be responsible for inhibited growth of most plants (Ashraf & Harris, 2004; Ashraf et al., 2010). For example, reduced leaf water potential in salt stressed pea plants has been reported to be due to higher Na⁺ and Cl⁻ accumulation (Noreen & Ashraf, 2009). In salt stressed turnip plants, decreased leaf relative water content and water potential resulted in inhibited plant growth (Noreen et al., 2010). Growth reduction due to salt-induced osmotic effect has been reported in a number of plants such as maize (Cramer et al., 1994), Brassica napus (Huang & Redman, 1995; Zheng et al., 1998), wheat (Kanwal et al., 2013), etc. In the present study, water relation parameters (leaf osmotic potential, turgor potential, water potential and relative water content) were adversely affected due to addition of excessive amount of salt to the root zone of all four rice cultivars. However, plants developed from nitric oxide treated seeds showed considerable improvement in water relation attributes under both control and saline conditions. Decreased water potential in the leaves of salt stressed plants has been reported mainly due to excessive accumulation of Cl⁻ and Na⁺ ions (Hasegawa et al., 2000; Flowers et al., 2010), which further leads to decreased leaf osmotic potential thereby reducing cell turgidity in plant cells due to water loss (Munns et al., 2002; Zhu, 2002; Ali & Ashraf, 2011). Although little information is available regarding the effect of exogenously applied nitric oxide on water relation attributes, however, according to Ke et al., (2013) nitric oxide treatment decreased solute potential while increased water potential in tobacco plants under osmotic stress.

Inorganic ion accumulation pattern is an important parameter for determining the level of plant salt tolerance (Ashraf, 2004; Munns & Tester, 2008). Plants try to avoid excessive accumulation of Na⁺ in the cytoplasm, because higher Na⁺ content interferes with normally occurring metabolic processes (Amtmann & Sanders, 1999). According to a number of reports, Na⁺ and Cl⁻ have been described as abundant components of saline soils and waters and their excessive amount in soil causes ion toxicity in plants which limits yield potential (Abrol et al., 1998; Munns & Tester, 2008; Habib et al., 2012). Although the role of excessive amount of Na⁺ in causing ion toxicity has been well documented, excessive Cl⁻ concentrations are also not desirable as Cl⁻ has been reported to cause more toxic effects in different crops including soybean, citrus, and grapevine (Lauchli, 1984; Ashraf, 1994; Grattan and Grieve, 1999; Storey & Walker, 1999). Cl⁻ has been reported to cause drying of leaf tissues in older leaves especially at extreme tips of leaf and cause leaf burn (Marschner, 1995). In the present study, salt stress resulted in excessive accumulation of Cl⁻ and Na⁺ while it decreased K' and Ca²⁺ accumulation in the leaf and root tissues of all four rice cultivars. NaCl-induced increase in Cl⁻ and Na⁺ content while a decrease in K' and Ca²⁺ accumulation have already been described in a number of crop plants such as okra (Habib et al., 2012), eggplant (Abbas et al., 2010), and wheat (Kanwal et al., 2013). Exclusion of excessive Cl⁻ and Na⁺ while maintenance of higher K'/Na⁺ ratio in plant tissues and cells have been reported to be key indicators to determine salt tolerance ability of crop plants (Zheng et al., 2009). NaCl induced decrease in K'/Na⁺ ratio could lead to impaired functioning of vital metabolic processes and activity of ROS scavenging system (Tester & Davenport, 2003; Munns & Tester, 2008). Balanced K'/Na⁺ ratio plays a key role in maintaining physiological processes such as stomatal regulation, cell osmoregulation, and turgor maintenance, which further helps in normal functioning of photosynthetic process and protein synthesis (Shabala et al., 2003). In the present study, exogenous application of nitric oxide application helped in decreasing the excessive Na⁺ accumulation, while it increased K' content and maintained higher K'/Na⁺ ratio in rice plants under salt stress. It has already been reported that exogenous nitric oxide treatment increases K' accumulation while decreases Na⁺ uptake thereby maintaining high K'/Na⁺ ratio in salt stressed plants of Arabidopsis (Zhao et al., 2007) and wheat (Zheng et al., 2009). These proactive roles of nitric oxide against salt-induced ionic effects have been reported due to increased activities of H⁺-PPase, H⁺-ATPase and Na⁺/H⁺ antiport in the tonoplast of nitric oxide treated plants (Zhang et al., 2006).

Conclusion

Overall, salt stress caused a significant effect on rice plants in terms of water relations and contents of inorganic ions. Imposition of salt stress significantly increased Na⁺ and Cl⁻ concentrations in the shoots and roots of rice plants which subsequently resulted in increased water and osmotic potentials while decreased leaf turgor potential. Salt-induced increase in Na⁺ concentration also caused a decrease in the uptake of Ca²⁺ and K⁺ in both shoots and roots of plants of all four cultivars. However, exogenous nitric oxide treatment (as pre-sowing seed soaking in SNP solution) effectively decreased the excessive Na⁺ uptake while improved K⁺ and Ca²⁺ concentrations which in turn resulted in improved water relation attributes in salt stressed plants of all four rice cultivars.

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Fig. 2. Shoot and root K⁺ and Ca²⁺ concentrations and K⁺/Na⁺ ratio in salt stressed and non-stressed four rice (Oryza sativa L.) cultivars when different levels of nitric oxide (NO) were applied as pre-sowing seed treatment.

References


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