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Protective effect of exogenousnitric oxide onalleviation ofoxidative damage induced by high salinity in rice (*Oryza sativa* L.) seedlings

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ABSTRACT-To find the protective role of exogenous nitric oxide (NO) on salinity-stressed rice seedlings, a CRD-based factorial experiment with three replications was conducted in Agronomy Laboratory of the Faculty of Agricultural Sciences, University of Guilan, in 2012. The experimental design consisted of healthy and vigorous seedlings of two rice cultivars, Khazar and Goohar, the last already known as promising SA13 line, which were exposed to 0 (Control), 50 mM NaCl, 50 µM sodium nitroprusside (SNP) as NO donor solution supplemented with simultaneous 50 mM NaCl + 50 µM SNP for four days. After 4 days, electrolyte leakage and malondialdehyde (MDA) content, activities of antioxidant enzyme, destruction of chlorophyll and soluble protein content in leaves were measured in treated and control plants. The results showed that simultaneous treatment of rice leaves with SNP, suppressed the ion leakage content by 8.5% compared with the results of NaCl treatment. Furthermore, SNPincreased the activities of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT). Exogenous application of NO also reduced peroxidation of membrane lipids, whereas increased the soluble protein content and chlorophyll pigments in rice leaves under salinity stress. These results suggested that NO could effectively protect rice seedlings from salt stress damaged by enhancing activities of antioxidant enzymes to quench the excessive reactive oxygen species caused by salt stress.

INTRODUCTION

Salinity has long been identified as one of the most pervasive environmental hazards, limiting crop production mostly in arid regions of the world (Ashraf and Harris, 2004). Salt stress can have a significant impact on the yield of all major cereals including rice, which is a salt sensitive species (Koyama et al., 2001). It has been understood that soil and water salinity has long beenidentified as a major issue for rice production (Greenland, 1984). High salinity environment can cause plant death or decrease productivity atthe whole-plant level. The loss in plant productivity is a consequence of imbalance inionic and nutrients concentration and osmotic effects (Ashraf, 2009) resulting in the overproduction of reactive oxygen species (ROS) such as superoxide($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2).

In plants, the antioxidant enzymes are important components in scavenging ROS (Ashraf, 2009). These enzymes have been identified in plants as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT). Previous studies have demonstrated that the antioxidant systems play important roles in protecting plants against oxidative damage induced by salt stress. It has been reported that salt stress caused significant increases of free radical production and membrane damage of rice resulting from decrease in the activity of antioxidant enzymes (Dionisio-Sese and Tobita, 1998). NaCl treatment also causes suppression of the growth of purslane seedlings due to a decreased antioxidant enzyme activity (Yaziciet al., 2007). Therefore, the use of techniques and/or methods to alleviate adverse effects of salinity stress is expected to result in sustainable crop productivity. However, the management of salinity stress has been considered difficult because of its multigenic and quantitative nature.Nitric oxide (NO) is an important signaling molecule exerting various physiological functions in plants (Zhenget al., 2009). It is reported that NO mediates the plants' responses to both biotic and abiotic stresses (Guo and Crawford, 2005). For instance, it involved seed germination (Beligni and Lamattina, 2000), affected the growth and development of plant tissue (Pagnussatet al., 2003), increased iron homeostasis (Durner and Klessig, 1999) and regulated plant maturation and senescence (Guo and Crawford, 2005). A few studies have already suggested that NO can play a role in protecting plants from oxidative stresses (Shiet al., 2005; Singh et al., 2008). (Li et al. 2008) found that SNP as an No-donor can effectively protect barley seedlings from salt stress damage by enhancing activities of antioxidant enzymes to quench ROS by salt stress. Furthermore, No-donor treatment has shown to increase the activities of SOD and CAT in the root of *Lupinus luteus* (Kopyra and Gwozdz, 2003), and to decrease contents of MDA and H_2O_2 in seed *Triticum aestivum* (Zheng et al., 2009) under salinity stress. However, little is known about the influences of exogenous NO treatment on oxidative damage and

antioxidant enzyme activities in leaves of rice under salt stress. Therefore, this study intends to investigate the effect of sodium nitroprusside (SNP), as an NO-donor, on protection responses of two rice cultivars under salt stress by enhancing the activity of antioxidant enzymes and improving physiological responses.

MATERIALS AND METHODS

Field Experiment

Seeds of two rice (*Oryza sativa* L.) cultivars, Khazar and Goohar, obtained from the Rice Research Institute of Iran, were surface-sterilized with 2.5% sodium hypochlorite for 10 min. Then, they were washed with sterile distilled water,and were placed on sterile filter paper moistened with deionized water and incubated at 27 °C for 72 h in dark to germinate. Germinated seeds were sown in plastic pots containing perlite and were allowed to grow in a growth chamber with 26/22 °C day/night temperature and 12/12 light/dark period at 350 μmol m^{-2} s^{-1} light density. The growth chamber daytime relative humidity was maintained 65±5%. Each pot was irrigated with 20 mL of Yoshida nutrient solution adjusted to pH 5.2 every three days.

NaCl and Nitric Oxide Treatment

Fifteen-day-old healthy and vigorous seedlings were selected for experimental treatments. The experimental treatments consisted of (Control; no added-SNP and –NaCl, 50 μM SNP, 50 mM NaCl, 50 mM NaCl + 50 μM SNP) and two rice cultivars (Khazar; very sensitive and Goohar; the last already known as promising SA13 line). Each treatment was replicated at least three times with 100 seedlings. At 4 days, all healthy leaves of uniform rice seedlings were collected and immediately preserved in liquid nitrogen and stored at −80 °C before assays biochemical characteristics.

Measured Parameters
Electrolyte Leakage:

The electrolyte leakage was determined in the manner described by Dionisio-Sese and Tobita(1998). Fresh rice leaves were cut into pieces of 5 mm length and placed in test tubes containing 10 mL distilled deionized water. The tubes were incubated in a water bath at 32 °C for 2 h and the initial electrical conductivity of the medium (EG_1) was measured. The samples were autoclaved at 121 °C for 20 min to release all electrolytes; cooled to 25 °C and the final electrical conductivity (EG_2) was measured. The relative electrolyte leakage (REL) was expressed as percentage and calculated by the equation:$REL= (EC_1/EC_2)\times 100$

Lipid Peroxidation:

The level of membrane damage was measured by determining malondialdehyde content (MDA) as the end product of membrane lipid peroxidation (Heath & Packer, 1968). Leaves were weighed and homogenized in a solution containing 10% trichloroacetic acid (TCA), and then centrifuged at 10,000 × *g* for 10 min. To 1.5 mL of the supernatant aliquot, 1.5 mL of 20% (w/v) TCA containing 0.5% (w/v) TBA, were added. The mixture was heated at 95 °C for 60 min and cooled to room temperature and centrifuged at 10,000 × *g* for 10 min. The absorbance of the supernatant was read at 532 and 600 nm against TCA solution as a reagent blank. The content of MDA was determined using the extinction coefficient of 1.55 M^{-1} cm^{-1} for MDA, and expressed in nmol MDA × g^{-1} of fresh weight (fr wt).

Enzyme Activity:

Rice leave samples (0.5 g) were homogenized in 1 mL of 50 mM potassium phosphate buffer, pH 7 containing 1 mM of EDTA in presence of polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 15,000 × *g* for 15 min at 4 °C. The supernatant was used to measure the activities of superoxide-dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and to determine total protein content. Enzymes activities were measured at 25 °C using a spectrophotometer (P.G Instrument T.80, England).

The activity of SOD (EC 1.15.1.1) was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) according to Giannopolitis and Ries's method. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 200 mM methionine, 1.125 mM NBT, 1.5 mM EDTA, 75 M riboflavin, and 0-50 μL of enzyme extract. Riboflavin was added as the last component. Reactions were carried out in test tubes at 25 °C under illumination supplied by two fluorescent lamps (20 W). The tubes were shaken before they were placed in the reaction assembly. The reaction was initiated by switching on the light and was allowed to run for 15 min, and switching the light off stopped the reaction. The tubes were then immediately covered with aluminum foil in order to stop the reaction and absorbance of the mixture which was then read at 560 nm. Identical tubes with complete reaction mixture, containing no enzyme extract, and developing maximum color served as a control. A non-illuminated complete reaction mixture with no color development served as blank. Under the experimental conditions, the initial rate of reaction, as measured by the difference in increase in absorbance at 560 nm in the presence and absence of leaf extract, was proportional to the amount of enzyme. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm.The SOD activity of the extract was expressed as activity unit/ g^{-1} fr wt.

POD (EC 1.11.1.7) activity in leaves was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Chance & Maehly, 1955). The reaction mixture contained 50 μL of crude enzyme extract, 500 μL of 5 mM H_2O_2, 500 μL of 28 mM guaiacol and 1950 μL of 50 mM potassium phosphate buffer (pH 7). POD activity of the extract was expressed as activity unit/ g^{-1} fr wt min^{-1}.

APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The reaction mixture in a total volume of 2 mL consisted of 25 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 50 mL enzymes extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm. The APX activity of the extract was expressed as APX unit/ g^{-1} fr wt. min^{-1}.

CAT (EC 1.11.1.6) activity was assayed according to Chance and Maehly's method (1955). The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm. The assay mixture contained 2.55 mL of 50 mM potassium phosphate buffer (pH 7.0), 400 µL of 15 mM H_2O_2 and 50 µL of enzyme extract. The CAT activity of the extract was expressed as activity unit/ g^{-1} fr wt. min^{-1}.

Chlorophyll and Protein Contents:

Chlorophyll (*Chl*) content was determined using Arnon's method (1949). 100 mg of the fresh leaf tissues were groundwithpestle and mortar in liquid nitrogen. Then, fine powder was transferred into tubes and 10 mL of absolute methanol was subsequently added to tubes and stored in the dark for 30 min. The homogenate was centrifuged at 5000 rpm for 15 min. The supernatant was used to measure the $Chl_a$and Chl_bcontent. 1 mL of the extract was then diluted with 9 mL of absolute methanol and the absorbance of the chlorophyll solution was measured using a spectrophotometer at 645 and 663 nm. The Chl_a and Chl_bcontent in µg were estimated using Arnon's formula.

Soluble protein content in leaves was determined according to using bovine serum albumin (BSA) as standard.

Statistical Analyses

The experiment was arranged in a completely randomized design with three replications. Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test mean comparison at $P<$ 0.05 using SAS software (v. 9.1, "SAS Institute", Cary, NC, USA). The figures were done using SigmaPlot® software (v. 12).

RESULTS AND DISCUUSION

The effect of NO Treatment onRelative Electrolyte Leakage and MDA Content:

There was no significant difference in electrolyte leakage content between the two cultivars of rice (Table 1). However, salinity and SNPtreatments had a significant effect on electrolyte leakage percentage in the rice leaves (Table 1). Salinity stress significantly induced rice leaves REL by 44% compared to the control group (Fig. 1). In the presence of NaCl and SNP together, the ion leakage decreased by 8% compared with the results of NaCl treatment (Fig. 1).

NaCl treatment resulted in the highest MDA content in both cultivars in comparison to control plants (Fig. 2). Under normal conditions, the application of SNP had a negative effect on MDA content compared to control treatment (Fig. 2). The addition of SNP significantly reduced NaCl-induced MDA accumulation after four-day salt stress-exposure (Fig. 2). It can be seen that SNP has a protective effect against NaCl-induced lipid peroxidation in rice leaves. Also, these results show a high rate of lipid peroxidation in Goohar cultivar due to salt stress (Fig. 2).

Table 1. Mean squares from analysis of variance of data for different biochemical attributes of two rice cultivars treated with or without SNP and NaCl for four days

SOV	d.f.	EL	MDA	SOD	POD	APX	CAT	PRT
Treatment (T)	3	59.4 **	4.60 **	832.6 **	23.2 **	160.6 **	0.210 **	177.5 **
Cultivar (C)	1	2.37 ns	1.92 **	42.7 ns	0.767 ns	18.9 **	0.031 **	97.7 **
T×C	3	1.56 ns	0.046 **	8.44 ns	1.16 ns	17.7 **	0.0004 *	15.2 **
Error	16	0.846	0.0003	54.6	1.11	0.308	0.0001	0.961

*, ** = significant at 0.05 and 0.01 levels, respectively; ns = non-significant. EL- electrolyte leakage; MDA- malondialdehyde; SOD- superoxide dismutase; POD- peroxidase; APX- ascorbat peroxidase; CAT- catalase; PRT- protein.

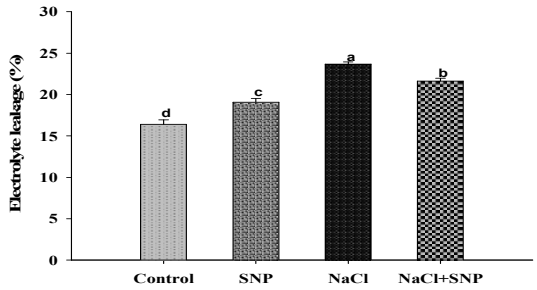

Fig. 1. Individual and combined effects of NaCl and SNP on the electrolyte leakage percentage in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at $P< 0.05$ (Duncan's multiple range test).

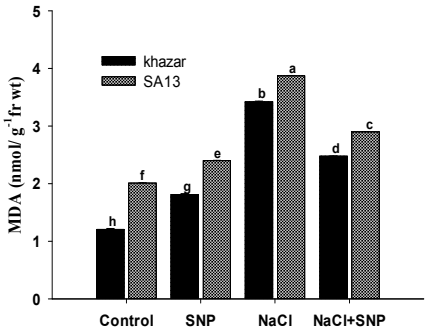

Fig. 2. Individual and combined effects of NaCl and SNP on MDA content in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at $P< 0.05$ (Duncan's multiple range test).

In the present study, we found that salt stress-induced oxidative damaged rice and measured the protective effect of exogenous NO. Salt stress caused increases in electrolyte leakage (Fig. 1) and MDA content (Fig. 2) in rice leaves. By generating changes in unsaturated fatty acids that affect membrane structure and properties, this electrolyte leakage and lipid peroxidation under salt stress may have also brought about an increase in membrane permeability or loss of membrane integrity (Dionisio-Sese and Tobita, 1998), and recuperation (though partial) upon NO supply indicates a preventive role of NO against NaCl-damage. In fact, NO prevents membrane damage by decreasing lipid peroxidation and electrolyte leakage from plant tissues (Li et al., 2008). However, the higher level of protection in plants treated with SNP may be a result of the more efficient antioxidant systems in SNP-treated plants. Shi et al. (2005) documented that NO reduces electrolyte leakage in bean leaves. Also, (Rubbo et al. 2000) reported that NO is a potent inhibitor of lipid peroxidation.A similar anti-oxidative function of exogenous NO was also observed in salt-stressed barley (Rubbo et al., 2000) and wheat (Zheng et al., 2009) plants. Therefore, exogenous SNP treatment could be an effective practice to protect rice seedlings against oxidative damage caused by salt stress.

The effect of NO Treatment on the Activities of Antioxidant Enzymes:

The activities of antioxidant enzymes significantly increased under NaCl treatment. The activities of SOD, POX, APX andCAT enzymes were shown in Fig. 3-6, respectively. There was no significant difference in SOD and POD activity between the two cultivars of rice (Table 1). However, SOD and POD activities were different among treatments (Table 1).

In the presence of NaCl and SNP together, the SOD activity was 56% higher than four-day NaCl treatment after exposure (Fig. 3); however, no significant difference was observed between cultivars. Also, under unsalinity conditions, the application of exogenous SNP did not greatly change SOD activity in rice leaves (Fig. 3).

SNP had induced a similar change at the POD activity and the maximal value of POD activity was 11.41 units g^{-1} fr wt. min at combined SNP + NaCl treatment, which was 25.2% more than NaCl treatment alone after four-day treatments (Fig. 4).

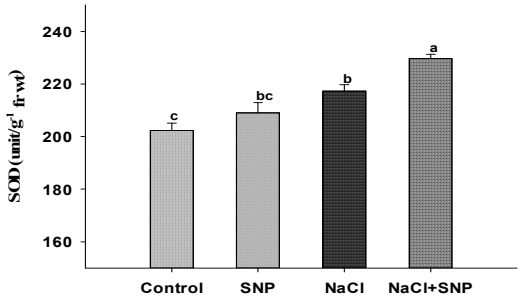

Fig. 3. Individual and combined effects of NaCl and SNP on SOD activity in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at p< 0.05 (Duncan's multiple range test).

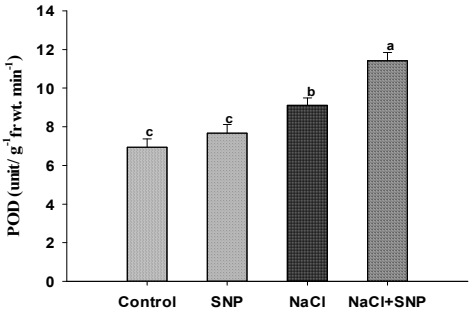

Fig. 4. Individual and combined effects of NaCl and SNP on POD activity in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at p<0.05 (Duncan's multiple range test).

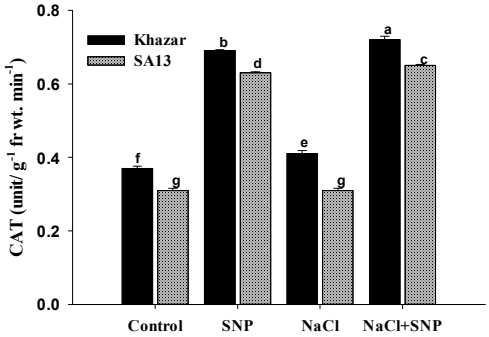

Fig. 6. Individual and combined effects of NaCl and SNP on AT activity in the rice leaves. Mean (±S.E.) was calculated Cfrom three replicates for each treatment. Different letters indicate significant differences at p< 0.05 (Duncan's multiple range test).

APX activity in rice leaves was significantly induced by salt stress (p< 0.05), and application of SNP had an obvious effect on APX activity (Fig. 5). Inducible effect of SNP on the activity of APX under stress conditions was higher than SOD and POD activities. Unlike SOD and POD, APX activity significantly increased in the two cultivars of rice (Fig. 5).

Similar to APX, NaCl stress significantly increased CAT activity in leaves of Khazar cultivar in comparison to its activity in control plants (Fig. 6). Under normal conditions, CAT activity was significantly promoted by SNP treatment (Fig. 6). The CAT activity was statistically different in the control plants and the plants treated with SNP and those treated with the combined treatment (NaCl and SNP) and the plants treated with NaCl only (Fig. 6).

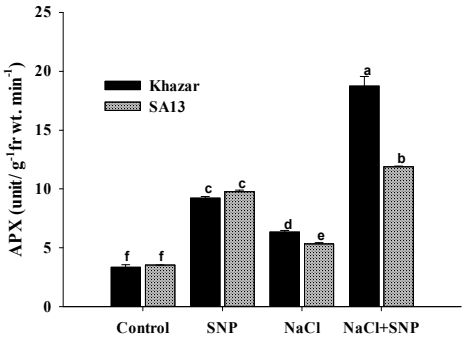

Fig. 5. Individual and combined effects of NaCl and SNP on APX activity in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at p< 0.05 (Duncan's multiple range test).

It is well known that the antioxidant enzymes such as SOD, POD, APX and CAT play a significant role in scavenging ROS in salt stressed plants (Ashraf, 2009; Tunaet al., 2008).Earlier studies have demonstrated that exogenous NO protects leaves against oxidative damage in reed under heat stress (Song et al., 2006) and in wheat under drought stress (Tian and Lei, 2006), which was mainly ascribed to the increased activities of SOD, POD and CAT. Thus, the protective role of NO may be attributed to its mediating the expression of genes encoding these ROS-scavenging enzymes under salt stress (Uchida et al., 2002; Li et al. 2008; Zheng et al., 2009). In accordance with these findings, we found that the activities of SOD (Fig. 3), POD (Fig. 4), APX (Fig. 5) and CAT (Fig. 6) in plants exposed to salt stress were significantlyincreased when NO was added, which was in agreement with theresults reported by Uchida et al. (2002), Kopyra and Gwozdz (2003), Li et al. (2008), and Zheng et al.(2009). These results confirmed that NO-donor addition can protect plants from damage caused by salt stress.

The effect of NO Treatment on Chlorophyll Content:

Chlorophyll *a*, chlorophyll *b* and total chlorophyll content significantly decreased in response to salinity stress (Table 2). The chlorophyll *a* (38.1%) and *b* (64.9%) levels and also total chlorophyll content increased in response to 50 Mm NaCl + 50 μM SNP (Table 2).

Chlorophyll loss has been reported as a criterion of senescence (Hung & Kao, 2003). Our results exhibited a decrease in chlorophyll content under NaCl treatment in rice seedlings, which hasalso been previously reported (Siringam et al., 2009). The decrease of leaf chlorophyll under salinity is due to the destruction of chlorophyll pigments and the instability of pigment protein complexes (Levitt, 1980). Adding SNP to the NaCl stressed seedlings markedly increased the chlorophyll content (Table 2). The increased chlorophyll content with the SNP treatment may be due to the ability of SNP to enhance cytokinin production, which stimulates chlorophyll biosynthesis (Fletcher et al., 2010).

Table 2. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and total chlorophyll (*TC*) in rice seedlings treated with or without SNP and NaCl for four days

Treatment	Chl_a (μg g^{-1} fr wt)	Chl_b (μg g^{-1} fr wt)	*TC* (μg g^{-1} fr wt)
control	7.71±0.258^{ab}	3.64±0.228^{a}	11.35±0.454^{b}
50 μM SNP	8.76±0.824^{a}	4.24±0.441^{a}	13.00±0.516^{a}
50 mM NaCl	5.96±0.112^{b}	2.34±0.315^{b}	8.30±0.643^{c}
50 mM NaCl + 50 μM SNP	8.19±0.764^{a}	3.86±0.293^{a}	12.05±0.300^{ab}
ANOVA	**	*	**

Mean (±S.E.) was calculated from three replicates for each treatment. Means followed by different letters are significantly different based on Duncan's multiple range test ($P< 0.05$). *, ** = significant at 0.05 and 0.01 levels, respectively.

The effect of NO Treatment on Protein Contents:

In this experiment, total soluble protein content reduced in the rice leaves under NaCl stress condition (Fig. 7). However, combined SNP + NaCl treatment increased the total soluble protein content in Khazar (53.1%) and Goohar (20/9%) cultivars compared with NaCl stressed plants (Fig. 7).

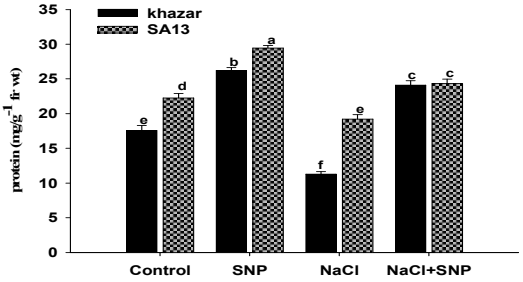

Fig. 7. Individual and combined effects of NaCl and SNP on protein content in the rice leaves. Mean (±S.E.) was calculated from three replicates for each treatment. Different letters indicate significant differences at p<0.05 (Duncan's multiple range test).

The reduction in protein content under salinity could be due to the decrease in protein synthesis, accelerated proteolysis, and decreased availability of amino acids and denaturation of enzymes involved in protein synthesis (Sharma and Dietz, 2009). SNP treatment in salt condition resulted in significantly higher leaf soluble protein accumulation for both cultivars which could probably be due to producing and identifying specific proteins (Key et al., 1981; Hoffman et al., 2012) that differentially accumulated in leaves. Among the different protein classes, we evaluated changes in different antioxidant enzymes due to previous research showing that salt stress could alleviate oxidative stress by improving ROS scavenging capacity (Mittler et al., 2004; Selote and KhannaChopra, 2010).

In this study, NO as an antioxidant, directly quenches the ROS and modulates various cellular physiological processes to limit oxidative injury and protect rice seedlings from salt stress damage (Uchida et al., 2002).

CONCLUSIONS

In conclusion, our study demonstrated that NO can effectively protect rice seedlings from salt stress damage by enhancing the activity of antioxidant enzymes and reducing membrane injury to prevent damage associated with excessive ROS. However, the application dose of NO donor needs further investigation according to different plant species and different growth stages.

REFERENCES

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1–15.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, 27, 84–93.

Ashraf, M., & Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166, 3-16.

Beligni, M.V., & Lamattina, L. (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta*, 210, 215-221.

Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-52.

Chance, B., & Maehly, S.K. (1955). Assay of catalase and peroxidases. *Methods in Enzymology*, 2, 764–775.

Dionisio-Sese, M.L., & Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Science*. 135: 1-9.

Durner, J., & Klessig, D.F. (1999). Nitric oxide as a signal in plants. *Current Opinion in Plant Biology*, 2, 369-374.

Fletcher, R.A., Gilley, A., Sankhla, N., & Davis, T.D. (2010). Triazoles as Plant Growth Regulators and Stress Protectants. In "*Horticultural Reviews*", pp. 55-138. John Wiley & Sons, Inc.

Giannopolitis, C.N., & Ries, S. K. (1977). Superoxide dismutases I. Occurrence in higher plants. *Plant physiology*, 59, 309-314.

Greenland, D.J. (1984). Exploited Plants: Rice. *Biologist*, 31, 291-325.

Guo, F.Q., & Crawford, N.M. (2005). Arabidopsis nitric oxide synthase 1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. *Plant Cell*, 17, 3436-3450.

Heath, R.L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125, 189-198.

Hoffman, L., DaCosta, M., Ebdon, J.S., & Zhao, J. (2012). Effects of drought preconditioning on freezing tolerance of perennial ryegrass. *Environmental and Experimental Botany*, 79, 11-20.

Hung, K.T., & Kao, C.H. (2003). Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. *Journal of Plant Physiology*, 160, 871-879.

Key, L.J., Lin, C.Y., & Chen, Y.M. (1981). Heat shock proteins of higher plants. *Proceedings of the National Academy of Sciences of the United States of America*, 78, 3526-3530.

Kopyra, M., & Gwozdz, E.A. (2003). Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology and Biochemistry*,41, 1011-1017.

Koyama, M.L., Levesley, A., Koebner, R.M.D., Flowers, T J., & Yeo, A.R. (2001). Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology*. 125: 406-422.

Levitt, J. (1980). Responses of plants to environmental stresses. Academic Press, New York. pp. 497.

Li, Q.Y., Niu, H.B., Yin, J.. Wang, M.B., Shao, H.B., Deng, D.Z., Chen, X.X., Ren, J.P., & Li, Y.C. Li. (2008). Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (*Hordeum vulgare*). *Colloids and Surfaces B: Biointerfaces*,65, 220-225.

Mittler, R., Vanderauwera, S., Gollery, M., & Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science*, 9, 490-498.

Nakano, Y., & Asada K. (1981). H_2O_2 is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22, 867-880.

Pagnussat, G.C., Lanteri, M.L., & Lamattina, L. (2003). Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiology*, 132, 1241-1248.

Rubbo, R., Radi, H.R., Anselmi, D., Kirk, M., Barnes, S., Butler, J., Eiserich, J.P. & Freeman, B.A. (2000). Nitric oxide reaction with lipid peroxyl radicals spares alphatocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha-tocopherol/ascorbate. *The Journal of Biological Chemistry*, 275, 10812-10818.

Selote, D.S., & KhannaChopra, R. (2010). Antioxidant response of wheat roots to drought acclimation. *Protoplasma*, 245, 153-163.

Sharma, S.S., & Dietz, K.J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science*, 14, 43-50.

Shi, S.Y., Wang, G., Wang, Y.D., Zhang, L.A., & Zhang, L.X. (2005). Protective effect of nitric oxide against oxidative stress under ultraviolet-B radiation. *Nitric Oxide*, 13, 1-9.

Singh, H.P., Batish, D.R., Kaur, G., Arora, K., & Kohli, R.K. (2008). Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environmental and Experimental Botany*, 63, 158–167.

Siringam, K., Juntawong, N., Cha-um, S., & Kirdmanee, C. (2009). Relationships between sodium ion accumulation and physiological characteristics in rice (*Oryza sativa* L.) seedlings grown under iso -osmotic salinity stress. *Pakistan Journal of Botany*, 41, 1837-1850.

Song, L., Ding, W., Zhao, M., Sun, B., & Zhang, L. (2006). Nitric oxide protects against oxidative stress under heat stress in the calluses from two ecotypes of reed. *Plant Science*, 171, 449-458.

Tian, X., & Lei, Y. (2006). Nitric oxide treatment alleviates drought stress in wheat seedlings. *Biologia plantarum*, 50, 775-778.

Tuna, A.L., Kaya, C., Dikilitas, M., & Higgs, D. (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany*, 62, 1-9.

Uchida, A., Jagendorf, A.T., Hibino, T. Takabe, T., & Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Science*, 163, 515-523.

Yazici, I., Türkan, I., Sekmen, A.H., & Demiral, T. (2007). Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. *Environmental and Experimental Botany*, 61, 49-57.

Yoshida, S., Forno, A.D., Cook, J.H., & Gomes, K.A. (1976). Laboratory Manual for Physiological Studies of Rice, (3rd ed.). The International Rice Research Institute, Los Banos, Laguna, Philippines.

Zheng, C., Jiang, D., Liu, F., Dai, T., Liu, W., Jing, Q., & Cao, W. (2009). Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environmental and Experimental Botany*, 67, 222-227.

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نقش حفاظتی نیتریک‌اکساید برون‌زاد در کاهش خسارت اکسیداتیو القاشده با شوری شدید در دانهال‌های برنج (*Oryza sativa* L.)

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چکیده–هدف این مطالعه، بررسی نقش حفاظتی نیتریک‌اکساید (NO) برون‌زاددر دانهال‌های برنج تحت تنش شوری بود. دانهال‌های قوی و سالم دو رقم برنج خزر و گوهر که پیشتر به‌عنوان لاین امیدبخش SA13 شناخته شده بود، با محلول ۵۰ میلی‌مولار نمک کلریدسدیم (NaCl)، ۵۰ میکرومولار محلول سدیم نیتروپروساید (SNP) و کاربرد هم‌زمان ۵۰ میلی‌مولار نمک کلریدسدیم و ۵۰ میکرومولار محلول SNPهمراه با شاهد (عدم کاربرد محلول) برای چهار روز تیمار شدند. پس از چهار روز، مقدار نشت یونی و مالون‌دی‌آلدهید (MDA)، فعالیت آنزیم‌های آنتی‌اکسیدانی، تخریب کلروفیل و مقدار پروتئین محلول در برگ‌های گیاهان تیمارشده و شاهد اندازه‌گیری شد. نتایج نشان داد که تیمار هم‌زمان برگ‌های برنج با SNP موجب کاهش مقدار نشت یونی در حدود ۸/۵ درصد در مقایسه با کاربرد نمک کلریدسدیم شد. علاوه براین، SNPسبب افزایش فعالیت آنزیم‌های آنتی‌اکسیدانی سوپراکسید دیسموتاز (SOD)، پراکسیداز (POD)، آسکوربات پراکسیداز (APX) و کاتالاز (CAT) شد. هم‌چنین، کاربرد NOبرون‌زاد موجب کاهش پراکسیده‌شدن لیپیدهای غشا، تأخیر در تجزیه پروتئین‌ها و تخریب رنگدانه‌های کلروفیل در برگ‌های برنج شد. این نتایج پیشنهاد می‌کند که NOمی‌تواند دانهال‌های برنج را به‌طور مؤثری از خسارت ایجادشده به‌وسیله تنش شوری با افزایش فعالیت آنزیم‌های آنتی‌اکسیدان در حذف گونه‌های فعال اکسیژن اضافی ناشی از تنش، محافظت کند.