Pak. J. Bot., 42(6): 4071-4078, 2010.

ENHANCEMENT IN SEED GERMINABILITY OF RICE (*ORYZA SATIVA* L.) BY PRE-SOWING SEED TREATMENT WITH NITRIC OXIDE (NO) UNDER SALT STRESS

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Abstract

The seeds of two fine-rice (Shaheen and PB-95) and two coarse rice (IRRI-6 and KS-282) cultivars were soaked in varying levels of nitric oxide (NO) (0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM) and then exposed to 80 mM NaCl in sand culture. Application of salt stress significantly reduced seed germinability parameters of all four rice cultivars in terms of percent seed germinated, germination index and seedling fresh and dry weights. The toxic effects of salt stress in reducing seed germinability were greater in fine rice cultivars (Shaheen and PB-95) as compared to those in coarse ones (IRRI-6 and KS-282). Although, the application of lower levels of nitric oxide (0.05, 0.1 and 0.2 mM) as pre-sowing seed treatment showed a significant improvement, 0.1 and 0.2 mM NO were found to be the most effective in improving seed germinability under salt stress. With a further increase in NO concentration (0.3 mM) as pre-sowing seed treatment, the seed germinability parameters differed non-significantly from those of control plants, while the highest levels (0.4 and 0.5 mM) showed significant inhibitory effects on seed germination and early seedling growth. It was concluded that lower levels of NO (0.1 and 0.2 mM) could be used to effectively enhance seed germination of rice plants under salt stress.

Introduction

Salt stress like many other abiotic stresses can considerably suppress growth and development of a number of plants (Maathuis, 2006; Läuchli & Grattan, 2007; Naz *et al*., 2010). Salt stress causes osmotic stress and ion toxicity in plants which further result in induction of oxidative stress and nutritional imbalances (Ashraf *et al*., 2010; Ashraf, 2010; Noreen *et al*., 2010). Most salt-affected soils consist of higher quantities of Na^+ and Cl^- ions derived from NaCl. These ions are highly toxic when they accumulate in high quantities as they damage the plant cells, inhibit growth and reduce yield (Läuchli & Grattan, 2007). Other toxic effects of salt stress include decreased germination and seedling growth (Ahmad *et al.*, 2010; Ashraf, 2010), and suppressed leaf expansion which ultimately reduces photosynthetic area and dry matter production (Mansour *et al*., 2005).

Nitrogen monoxide (NO), most commonly known as nitric oxide is a lipophilic free radical and volatile in nature (Hayat *et al*., 2010). It acts as important signaling molecule involved in regulation of a number of physiological processes in animals as well plants (Crawford & Guo, 2005; Besson-Bard *et al*., 2008). In earlier studies, the regulatory roles of NO have been reported at different stages of crop development and have been especially found beneficial in promoting seed germination of most plant species (Bethke *et al.*, 2007; Libourel *et al.*, 2006). In addition to its regulatory role in plants NO has been reported to confer stress tolerance against a variety of biotic and abiotic stresses, such as drought (Garcia-Mata & Lamattina, 2002) and salt stress (Zhao *et al*., 2007). Therefore, plant stress tolerance can be increased by external application of donors of NO (Zhao *et al*., 2007). For example, Zhang *et al.,* (2006) reported that exogenous application of NO as pre-sowing treatment to maize seeds resulted in enhanced salt tolerance ability of maize seedlings.

Since NO has been reported to involve in regulation of a variety of physiological processes in plants particularly under salt stress, it can be assumed that increased level of endogenous NO by exogenous supplementation can enhance salt tolerance of rice. Therefore, the main objective of the present study was to assess that up to what extent exogenous application of NO could enhance the seed germinability parameters of rice under salt stress because rice is generally known as a salt-sensitive crop (Zeng *et al*., 2003; Ahmad *et al*., 2007).

Materials and Methods

Seeds of four rice cultivars were obtained from the Rice Research Institute, Kala Shah Kaku (KS-282 and IRRI-6) and Soil Salinity Research Institute, Pindi Bhatian (Shaheen basmati and PB-95). Of these four, Shaheen basmati and Basmati PB-95 were fine-rice and IRRI-6 and KS-282 coarse-rice type. Seeds of all four cultivars were surface sterilized with 0.1% mecuric chloride for one minute and soaked in varying solutions of sodium nitroprusside (nitric oxide donor) (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mM) for 16h as pre sowing treatment. The control seeds were soaked in distilled water only. Ten seeds of uniform size from each cultivar were sown in small plastic pots containing 0.5 kg sand. Two levels of NaCl (0 and 80 mM) were prepared in Hogland's nutrient solution and 200 ml of each treatment solution were applied to each pot before sowing. The pots were arranged in a completely randomized design with three replicates. The pots were placed in a net-house under natural conditions (*PPFD* 1275 µmol m^{-2} s^{-1}; average day and night temperatures 36±3°C and 27±2°C, respectively; relative humidity 44.1%, and day-length 13.7 h). The seeds were allowed to germinate under these conditions and number of seeds germinated was counted every-day until five days. A seed was considered germinated when both plumule and radicle had emerged ≥ 0.5 cm. The data so obtained was used for the calculation of seed germination percentage, germination index and time to achieve 50% germination (in days). Seedlings were harvested after 12 days of sowing, washed with distilled water and fresh weight of plumule recorded. The seedlings were wrapped in a paper bag, dried in an oven at 65°C to a constant dry weight and dry weights recorded. Data of various seed germiniability and seedling growth parameters were analyzed statistically by analysis of variance technique (Steel & Torrie, 1986) based on two-factor factorial in a completely randomized design (CRD) using Costat computer package (CoHort Software, 2003, Monterey, California).

Results

Application of salt stress had a significant effect on seed germination in terms of germination percentage, germination index, days to achieve 50% germination and plumule fresh and dry weights. Pre-sowing seed treatment with NO particularly its lower levels significantly alleviated the toxic effects of salt stress while higher concentrations of NO adversely reduced all these seed germinability parameters. Statistical analysis of the data for germination percentage revealed highly significant differences for NO, salinity and cultivar means. Although. NO x S, interaction term was also significant, NO x C, S x C and NO x S x C interaction terms differed non-significantly for percent germination of rice cultivars (Table 1). It was observed that lower level of NO application (0.05 mM) had a slight improvement in seed germination in both basmati rice cultivars (Shaheen and PB-95), but had no effect on percent germination of coarse rice cultivars (IRRI-6 and KS-282). However, with a further increase in NO concentration as a pre-sowing seed treatment (0.1 and 0.2 mM), seed germination was significantly improved in all four cultivars being more effective in basmati rice cultivars as compared to that in coarse ones. A non-significant difference was observed at 0.3 mM level of NO in all four rice cultivars with that of control plants. The higher levels of NO application (0.4 and 0.5 mM) significantly decreased seed germination of all four cultivars either under control or saline conditions. Overall, NO-induced improvement in seed germination was greater under saline conditions than that under control conditions (Fig. 1).

Seed germination index was significantly improved by pre-sowing seed treatment with NO and lower levels being more effective under both normal and saline conditions (Table 1). Although, the lower level of NO (0.05 mM) application showed a slight increase in seed germination index, the extent of improvement was more due to application of 0.1 and 0.2 mM NO in all four cultivars under both control and saline conditions. The four cultivars did not differ significantly in seed germination index with a further increase in NO level (0.3 mM) under control as well as saline conditions. However, at the highest NO levels (0.4 and 0.5 mM) germination index was severely reduced in all four cultivars. Under normal conditions, cv. Shaheen basmati exhibited a maximum improvement in germination index with exogenous NO application followed by IRRI-6, PB-95 and KS-282, respectively. However, under saline conditions, IRRI-6 showed a maximum germination index in all NO treatments followed by Shaheen basmati, PB-95 and KS-282, respectively (Table1; Fig. 1)

Application of salt stress significantly delayed the time to achieve 50% germination but pre-sowing seed treatment with NO significantly reduced it particularly at the lower levels of NO. The 50% germination time was significantly reduced by the application of lower levels of NO under both control and saline conditions being 0.05, 0.1 and 0.2 mM NO level the most effective in reducing this time period in coarse cultivars (IRRI-6 and KS-282). With a further increase in NO level (0.3 mM) the time to achieve 50% germination increased significantly and the maximum delay in achieving 50% germination was observed at the highest levels (0.4 and 0.5) of NO application. Overall, both coarse rice cultivars (IRRI-6 and KS-282) took significantly less time to achieve 50% germination than that of fine rice cultivars Shaheen and PB-95 (Table1; Fig. 1).

Plumule fresh weight in all four rice cultivars decreased significantly by the application of salt stress. The lower level of NO application (0.05 mM) did not show a significant effect on Shaheen and PB-95 under control conditions but this level significantly increased plumule fresh weight under saline conditions in the both fine cultivars. As compared to fine rice cultivars, 0.05 mM NO application significantly enhanced plumule fresh weight in both coarse rice cultivars (IRRI-6 and KS-282) under both control and saline conditions. With a further increase in exogenous NO levels (0.1 and 0.2 mM) plumule fresh weight increased consistently in all four cultivars under saline conditions. A further, increase in NO level (0.3 mM) did not significantly improve plumule fresh weigh, whereas and 0.4 and 0.5 mM NO levels significantly reduced plumule fresh weight under both growth conditions in all four cultivars (Table 1; Fig. 2).

Table 1. Mean squares from analysis of variance of the data for seed germinability and early seedling growth parameters

Sources of variation	d.f.	Germination (%)	Germination index	Time to 50% germination (t_{50})	Plumule f. wt.	Plumule d. wt.
Nitric oxide (NO)	6	1440.07***	53.85***	1.95***	0.001***	5.64***
Salinity (S)	1	2288.09***	136.62***	7.51***	0.005***	6.43***
Cultivar (C)	3	1579.36***	52.75***	3.97***	1.291***	4.46***
Interactions						
NO x S	6	129.76*	2.92**	0.06ns	8.988***	8.80ns
NO x C	18	26.58ns	3.31***	0.13***	1.620ns	1.16*
S x C	3	96.03ns	3.27**	0.45***	1.212***	5.71ns
NO x S x C	18	76.58ns	2.95***	0.03ns	2.030ns	5.89ns
Error	**112**	**55.35**	**0.73**	**0.03**	**2.083**	**6.19**

Similar to the reduction in plumule fresh weigh, plumule dry weight was also significantly decreased by the imposition of salt stress. The lower level of NO application (0.05) showed some improvement, whereas 0.1 and 0.2 mM levels showed highly significant improvement in plumule dry weight in all four rice cultivars under both control and saline conditions. However, at all these levels of NO application, the performance of coarse rice cultivars was better as compared to that of fine ones. Further increase in NO level (0.3 mM) did not show any significant effect on plumule dry weight under saline conditions as compared to that under control conditions. The highest levels of NO (0.4 and 0.5 mM) application as a pre-sowing seed treatment had a significant inhibitory effect on plumule dry weights in all four rice cultivars being more toxic in fine rice cultivars as compared to that in coarse rice cultivars (Table 1; Fig. 2).

Discussion

Seed germination and early seeding growth of rice has been shown to be highly sensitive to salt stress because of marked effects of osmotic stress and specific ion toxicity on these growth stages in this plant species (Mohammad & Sen 1990; Duan *et al.*, 2004). In the present study, salt stress severely affected various seed germination attributes including germination percentage, germination index and time to achieve 50% germination in all four rice cultivars (Shaheen, PB-95, IRRI-6 and KS-282). In addition, plumule fresh and dry weights were also significantly reduced in all four rice cultivars under salt stress. However, the lower levels of NO (0.1 and 0.2 mM) applied as pre-sowing seed treatment significantly increased the germination percentage in all four cultivars. It has been reported that Sodium nitropruside (SNP) functions as a NO donor (Bethke *et al.*, 2004). NO, in fact is an important signaling molecule involved in the regulation of a range of physiological processes in animals as well as plants (Crawford & Guo, 2005; Besson-Bard *et al.*, 2008). Therefore, due to its regulatory roles it can promote seed germination of various crop plants including rice under stressful conditions (Bethke *et al.*, 2007; Libourel *et al.*, 2006). For example, Zheng *et al.*, (2006) reported that NO significantly increased dry matter accumulation in maize seedlings under salt stress. Later some authors reported that seed germination rate and weight of coleoptile and radicle were significantly increased with NO application in wheat seedlings under salt stress (Zheng *et al.*, 2009). All these reports indicate that exogenous application of NO as a pre-sowing seed treatment significantly enhances salt stress tolerance potential of different crops at early growth stages i.e. germination and seedling stage.

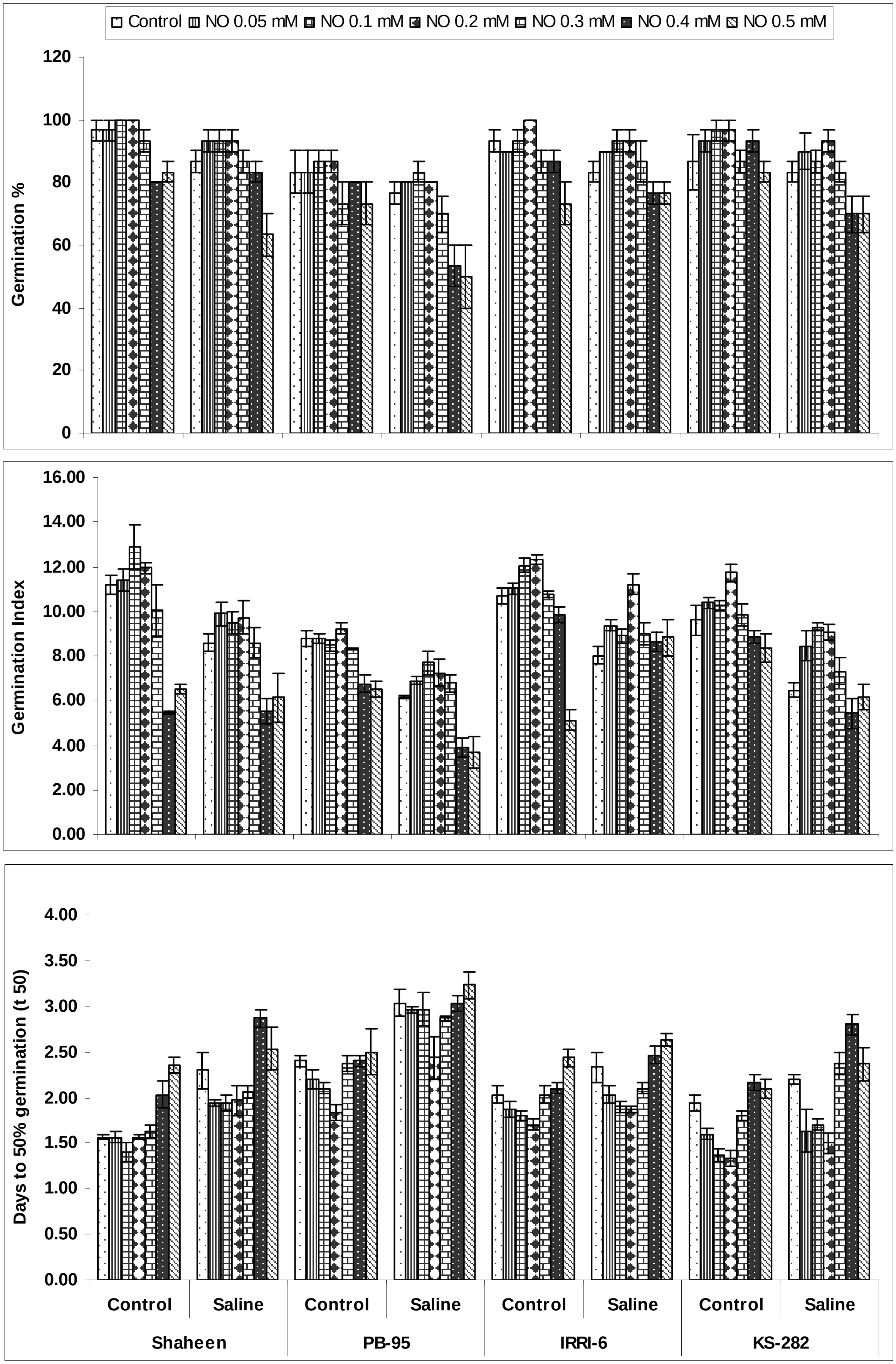

Fig. 1. Effect of various levels of NO on germination percentage (a), index (b) and days to 50% germination (t_{50}) of two fine (Shaheen and PB-95), and coarse (IRRI-6 and KS-282) rice cultivars.

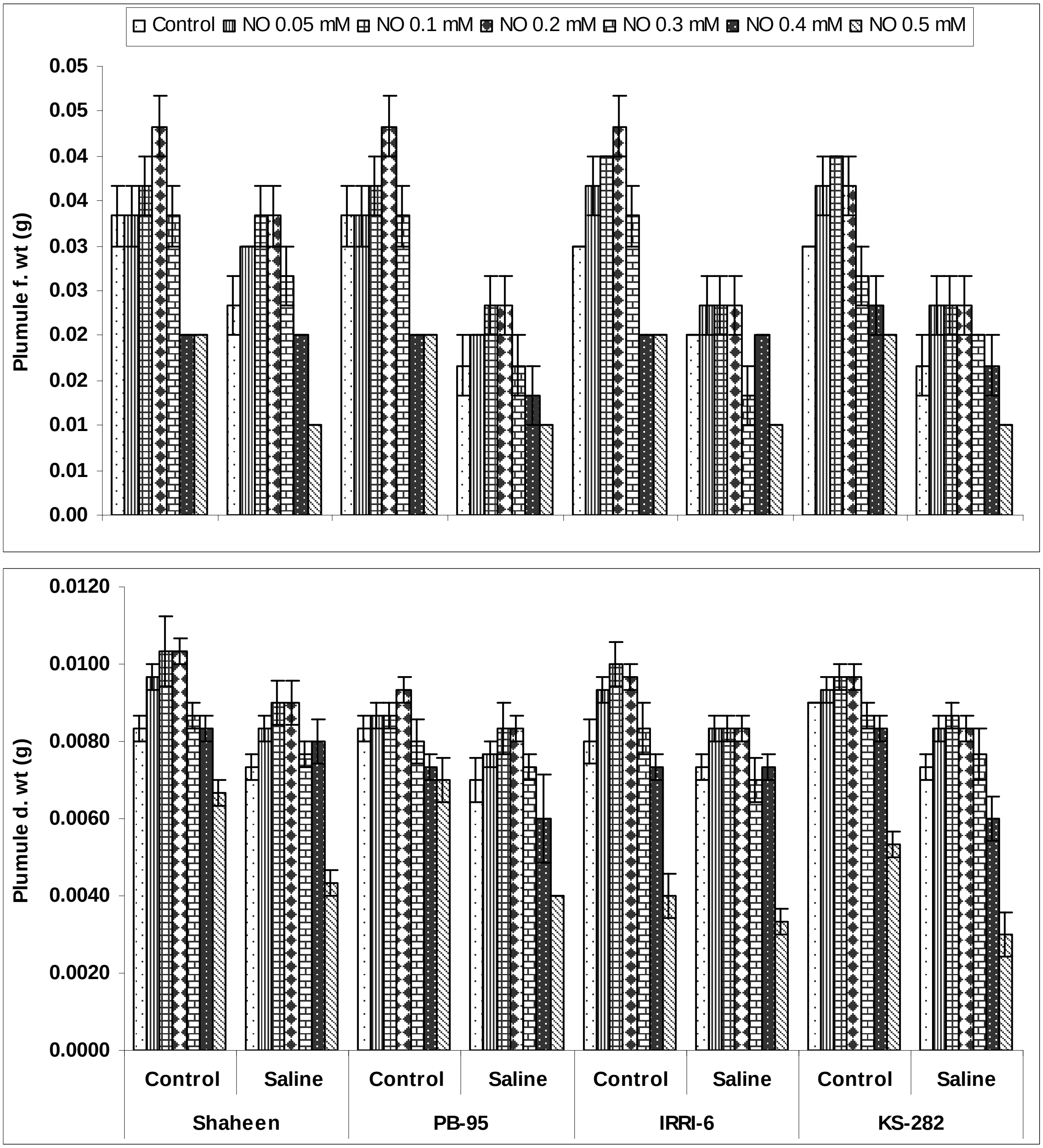

Fig. 2. Effect of various levels of NO on plumule fresh (a) and dry (b) weights of two fine (Shaheen and PB-95), and coarse (IRRI-6 and KS-282) rice cultivars.

Application of NO in high concentration (0.4 and 0.5 mM) as pre-sowing seed treatment severely reduced seed germinability parameters under normal as well as saline conditions. This was true for both fine as well as coarse rice cultivars but the extent of reduction was less in coarse cultivars than that in fine ones. The high levels of NO have been reported to reduce seed germination and seedling growth of a number of crop plants (Zhang *et al*., 2003; Kopyra & Gwozdz, 2003). The toxic effect of NO in high concentrations has most widely been attributed to its ability to damage membranes and cause DNA fragmentation (Pedroso *et al*., 2000; Yamasaki, 2000; Romero-Puertas *et al*., 2004). In addition, high concentrations of NO may cause arrest of cell division, apoptosis, and ultimately senescence of whole plants. It has also been reported that higher concentration of NO inhibits the activity of some potential anti-oxidative enzymes such as catalase leading to higher H_2O_2 and other reactive oxygen species (ROS) (Bethke *et al*., 2007). All these phenomena may have led to reduced germination of rice under high NO application as observed in the present study.

Conclusion

Salinity stress significantly reduced different seed germinability parameters in fine-rice cultivars as compared to coarse ones. Application of lower levels of NO (0.1 and 0.2 mM) significantly alleviated the toxic effects of salt stress on early growth stages of all rice cultivars. However, 0.05 and 0.3 mM NO levels had non-significant effect on seed germination parameters. The higher levels of NO (0.4 and 0.5 mM) adversely reduced all parameters recorded in this study. It can be concluded that pre-sowing seed treatment with 0.1 and 0.2 mM NO can be effectively used to alleviate the adverse effects of salt stress on seed germination of the rice crop.

Acknowledgement

The data presented in this paper is a part of Ph.D. research studies of Mr. Noman Habib (2003-ag-491), a HEC scholar at the Department of Botany, University of Agriculture, Faisalabad.

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(Received for publication 12 November 2009)