



Full Length Article

Cross Adaptation Tolerance in Rice Seedlings Exposed to PEG Induced Salinity and Drought Stress

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Abstract

The present study investigated the effects of polyethylene glycol (PEG) on rice (*Oryza sativa* L.) seedlings for physiological and biochemical response exposed to subsequent salinity or polyethylene glycol and NaCl (as drought) stress. Salinity or drought induced stress decreased growth-significantly ($p < 0.05$) reduced pigment content, stomatal conduction (g_s), transpiration (E) and net photosynthetic rate (P_n), whereas no significant change in intercellular CO₂ concentration (C_i) was recorded relative to controls. NaCl+PEG stress also reduced POD activity. MDA content and total soluble sugar content increased significantly ($p < 0.05$) under NaCl or NaCl+PEG induced stress. Chlorophyll fluorescence decreased significantly in NaCl-treated plants. However, positive effects were observed in PEG pre-treated (PEG-NaCl, PEG-NaCl+PEG) rice seedlings than NaCl or NaCl+PEG-treated ones. PEG pre-treatment promoted rice seedlings growth and regulation capacity in rice seedlings tolerance to NaCl or drought stress by increase in plant biomass, chlorophyll contents, chlorophyll fluorescence, photosynthetic parameters and antioxidative enzymes activities. The positive effects to the pre-treatment of rice seedlings suggested that cross-adaptation of PEG pre-treatment mediated protection of salinity and drought stress. © 2016 Friends Science Publishers

Keywords: Polyethylene glycol; NaCl; Rice; Antioxidative enzymes; Malondialdehyde

Introduction

Salinity stress is a severe factor limiting agricultural crop production in arid and semi-arid regions. Furthermore, increasing salinity is threatening human survival soil resources. It has been reported that due to salt stress up to 20% of the irrigated land has been affected (Abogadallah, 2010). Drought stress is also another abiotic stress limiting crop production. However, most of studies report the effects of salinity and drought stress on plant growth as individual factor (Malash and Khatab, 2008; Hao *et al.*, 2012). These studies clearly explain mechanics of plant growth under salinity or drought single stress factor, however under the field condition, the co-incidence of salinity and drought stress always exist, which may lead to plants suffering simultaneously from multiple abiotic stresses. Therefore, studies of the relationships between salinity and drought stress would be more closely approximate to natural conditions.

Both salinity and drought stress have profound effects on plant physiology. Each of these stresses induces stomatal closure; decrease chlorophyll contents and reduce photosynthesis (Nemeth *et al.*, 2002; Ma *et al.*, 2012). It also disrupts cell membrane function and elicits lipid peroxidation, protein degradation, and disturbs redox homeostasis by forming reactive oxygen species (ROS)

(Mühling and Läuchli, 2003). Plants under salinity and/or drought environments have developed several mechanisms to adapt to abiotic stresses relying on different physiological mechanisms that include cellular osmotic adjustments, and/or antioxidant systems. Antioxidant systems consist of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), either constitutive or inducible. Osmoprotector compounds such as soluble sugar also play important role in response to salinity and/or drought stress (Sharma and Dietz, 2006).

Rice (*Oryza sativa* L.) belongs to sensitive crop to salt stress. Salinity stress is a significant factor in reducing rice productivity. Researchers are looking for effective method to reduce salt damage to plants. For example, transgenic technology was applied to increase crop resistance to salt stress (Upadhyaya *et al.*, 2011) and traditional breeding techniques to breed salt-tolerant varieties (Phang *et al.*, 2008). And some cultural techniques such as drought pre-treatment applied at seedling stage enhanced salt tolerance in adult tomato plants (Malash and Khatab, 2008). Plants often produce tolerance to subsequent other stresses “cross-adaptation” when early exposed to moderate stress (Boussiba *et al.*, 1975). The plant genome regulated the adaptive process and specific proteins induced by first stress protect against sequential stress in case of cross-adaptation (Sabehat *et al.*, 1998). Furthermore, pre-treatment plants

could keep the adaptability during the growth period (Cuartero *et al.*, 2006).

To investigate the effects of polyethylene glycol (PEG) pre-treatment on NaCl and NaCl+PEG stress, it was used to stimulate water stress in rice seedlings. Pigment content, photosynthetic parameters, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA) content, and soluble sugar content were analyzed to elucidate the possible mechanisms. Using PEG as pre-treatment may present an interesting strategy for mitigating salinity/salinity and drought tolerance in rice.

Materials and Methods

Plant Culture and Treatments

Rice (*Oryza sativa* cv. Liaoxing No. 1) seeds were surface sterilized in 2% sodium hypochlorite solution for 10 min and rinsed three times in sterile distilled water, then soaked in sterile deionized water at 28°C for 6 h. The seeds were germinated at 28°C for 48 h in the dark. Next day, the germinated seeds were grown hydroponically in Hoagland's nutrient solution in a growth chamber at a day/night cycle of 16 h/8 h, at 27°C/20°C, respectively with 80% relative humidity. The nutrient solution was renewed every 2 days.

After growing 7 days of rice seedlings, 10% PEG-6000 was added to the nutrient solution for 3 days. Then 100 mM NaCl or 10% PEG + 100 mM NaCl was added to the nutrient solution for another three days. Thus, five treatments were established - including control, NaCl alone stress, 10% PEG + NaCl combined stress, PEG-NaCl pre-treatment and PEG-NaCl+PEG pre-treatment. All treatments were conducted in three replicates.

Growth Parameters

Seedling height, root length and fresh weight were recorded at 7th day after pre-treatment. Plant dry weight was determined at 80°C until a constant dry weight.

Total Chlorophyll, Photosynthetic and Chlorophyll Fluorescence Measurements

Fresh 0.1 g of second leaf was used to extract total chlorophyll ($a+b$) and carotenoid (Car) content in 10 mL 80% cold acetone and ethanol (v/v =1:1). Then measured spectrophotometrically following Lichtenthaler's method (1987).

Photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO₂ concentration (C_i) and transpiration rate (E) were measured using a portable photosynthesis system (LI-6400, Lincoln, NE, USA) in the open circuit mode between 10:00 and 12:00 at 7th day after pre-treatment.

Minimal chlorophyll fluorescence (F_0) and maximal chlorophyll fluorescence (F_m) were measured using LI-6400-40LCF (LI-COR, Lincoln, NE, USA) following the method of Strasser and Srivastava (1995). Photosystem II efficiency parameter F_v/F_m was calculated according to the formula $F_v/F_m = (F_m - F_0)/F_m$. The values of F_v/F_m and F_v/F_0 reflected the maximal quantum yield of photosystem (PS) II photochemistry and the potential activity of PS II, respectively.

Antioxidant Enzyme Activities

After 6 d treatment, enzymes were extracted at 4°C from 0.5 g leaves in mortar and pestle with 5 mL of extraction buffer, containing 0.1 M phosphate buffer (pH 7.8), 0.1 mM EDTA, 1% PVP. Extracts were centrifuged at 10,000×g for 15 min, and the supernatants were used as the crude extract for antioxidative enzymes analysis. SOD activity was assayed by the inhibition of the photochemical reduction of β -nitro blue tetrazolium chloride (NBT). One unit of SOD was defined as the amount of enzyme, which produced 50% inhibition of NBT reduction at 560 nm (Costa *et al.*, 2002). CAT activity was determined that the decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm (Cakmak and Horst, 1991). POD activity was measured with guaiacol as the substrate. Increase in the absorbance due to oxidation of guaiacol was measured at 470 nm (Kochba *et al.*, 1977). To calculate specific enzyme activities, protein concentration was estimated according to the method of Lowry *et al.* (1951).

Total Soluble Sugars

Fresh 0.5 g leaves were collected to determine total soluble sugars as described by Irigoyen *et al.* (1992). Samples were homogenized in 5 mL of 95% (v/v) ethanol and the homogenate was centrifuged at 3 000 × g for 5 min. 0.1 mL supernatant was added to 0.4 mL freshly prepared anthrone reagent at 100°C for 10 min and cooled to room temperature in an ice bath. The soluble sugar content was measured spectrophotometrically (721, Shanghai analytical instrument factory, Shanghai) at 630 nm. Glucose was used to as standard.

Lipid Peroxidation

The estimation of MDA content was determined following by Heath and Packer (1968). Fresh 1.0 g leaves were ground with 4 mL of 0.1% (w/v) trichloroacetic acid (TCA) in ice bath. The homogenate was centrifuged at 10,000×g for 5 min. One mL (1 mL) aliquot of the supernatant was reacted with four milliliter of 0.5% thiobarbituric acid (TBA) in 20% TCA at 100°C for 30 min and cooled quickly to room temperature. Then, MDA content was determined using spectrophotometer at 532 nm and corrected for unspecific turbidity at 600 nm.

Statistical Analysis

All data were analyzed taking $p < 0.05$ as significance according to Tukey's multiple range test using the SPSS 11.0 software package. The results were shown as mean \pm standard deviation (SD).

Results

Seedling Growth and Biomass Accumulation

There was no significant difference in seedling height among treatments. Root length, fresh and dry weights were markedly inhibited for plants exposed to 100 mM NaCl compared with the controls. The NaCl+PEG stress led to a further significant reduction in root length compared with NaCl alone. That the pretreated plants had higher values of growth parameters compared to non-pretreated plants exposed to NaCl and NaCl+PEG stress.

Effects on Photosynthetic, Chlorophyll Fluorescence and Total Chlorophyll

Net photosynthetic rate (P_n) decreased obviously ($p < 0.05$) for all treatments, however PEG pre-treatment had a significant ($p < 0.05$) effect on the P_n of plants exposed to NaCl or NaCl+PEG stress in comparison with the controls (Fig. 2a). Stomatal conductance (g_s) showed also a significant ($p < 0.05$) decrease following treatments of NaCl or NaCl+PEG stress, with no apparent difference under pre-treatment (Fig. 2b). There was the same trend for transpiration (E) as for g_s (Fig. 2d). Internal CO₂ concentration (C_i) showed no significant effects on all treatments in comparison with controls (Fig. 2c).

The NaCl treatments significantly decreased F_v/F_m and F_v/F_0 , whereas the NaCl+PEG treatments and PEG pre-treatments showed no significant effects compared with the controls (Fig. 3). There was a significant change between PEG pre-treatment and NaCl stress, but not NaCl+PEG stress.

Total chlorophyll content in NaCl or NaCl+PEG stress treatments significantly decreased in comparison with the controls, and was significantly lower than of pre-treated plants (Fig. 4a). Carotenoid content were also lower in NaCl or NaCl+PEG-treated plants than in control plants (Fig. 4b). However, carotenoid content significantly increased in pre-treated plants compared with plants exposed to NaCl or NaCl + PEG stress. But there were no significant difference between the pre-treated plants and the control plants in carotenoid content.

Antioxidant Enzyme Activities

A comparison of antioxidative enzyme activities such as SOD, POD and CAT in pre-treated and non-pretreated plants subjected to NaCl or NaCl+PEG stress is depicted in Fig. 5.

SOD activity showed marked increase in plants exposed to NaCl and NaCl+PEG. In pre-treated plants, SOD activity increased notably ($p < 0.05$) in leaves compared with NaCl-treated plants, but not NaCl+PEG-treated plants (Fig. 5a). Exposed to NaCl-treated plants, POD activity was higher than controls. In pre-treated plants, POD activity was significantly lower than NaCl and NaCl+PEG stress (Fig. 5b). CAT activity significantly ($p < 0.05$) increased subjected to NaCl stress, with significant changes in pre-treated plants (Fig. 5c). Exposure to NaCl+PEG stress, CAT activity was higher than controls, but showed no significant effects (Fig. 5c).

Total Soluble Sugar

Total soluble sugar content was markedly higher exposed to NaCl or NaCl+PEG stress than the controls (Fig. 6). The pre-treated plants showed higher total soluble sugar content than non-pretreated plants.

Lipid Peroxidation

MDA content, as the level of lipid peroxidation, had a marked ($p < 0.05$) increase in both pre-treated plants as well as non-pretreated plants compared to the controls (Fig. 7). The pre-treated plants showed significant lower MDA content than non-pretreated plants (Fig. 7).

Discussion

Cross-adaptation tolerance is found in some plants due to subsequent other stresses when early exposed to moderate stress. For example, moderate salinity stress alleviated following cadmium stress in rice (Ma *et al.*, 2013), salt stress in mungbean (Saha *et al.*, 2010) and copper toxicity in *Mesembryanthemum crystallinum* (Volkov *et al.*, 2006). Water stress induced salt resistance in sugarcane (Neelma and Faheem, 2009) is also reported.

Salinity stress inhibited plants growth as well as water stress (Munns, 2002). Pronounced damaging effects occurred in rice plants exposed to NaCl or NaCl+PEG stress, also indicated with reduced growth prominently compared to controls (Fig. 1). If growth reduction is considered as damaging consequence during plants development processes, growth increase will be considered as morphological adaptation to adverse factors. In this study, we observed that PEG pre-treated plants showed growth increase in all growth traits. This showed PEG pre-treatment partially alleviated adverse effects on rice plants exposed to NaCl or NaCl+PEG stress.

Shahbaz *et al.* (2011) reported that salt induced decline in sunflower growth is due to reduction in photosynthetic capacity. In this study, apart from growth, photosynthetic rate (P_n) showed a significant ($p < 0.05$) decrease subjected to NaCl or NaCl+PEG stress (Fig. 2a). This consistently matched well to the changes in DW and FW of the plants under NaCl or NaCl+PEG stress (Fig. 1).

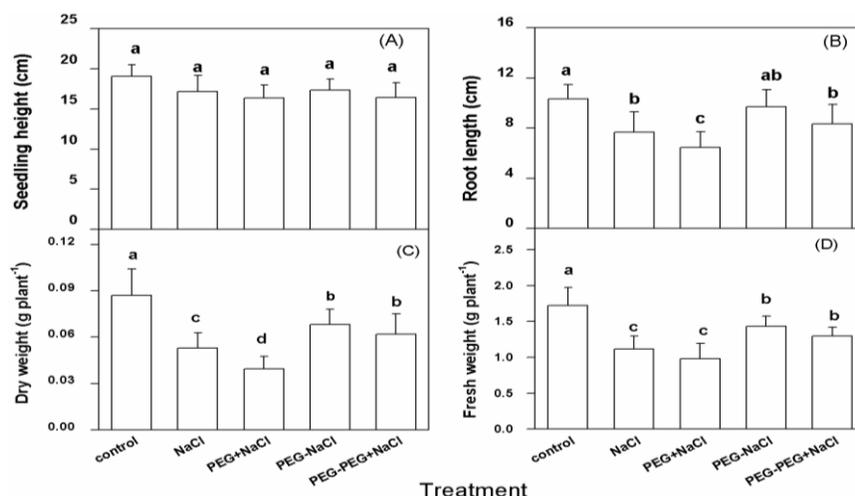


Fig. 1: Effects of PEG pre-treatment on growth parameters of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents means \pm SD. The different letters indicate significant differences at $p < 0.05$

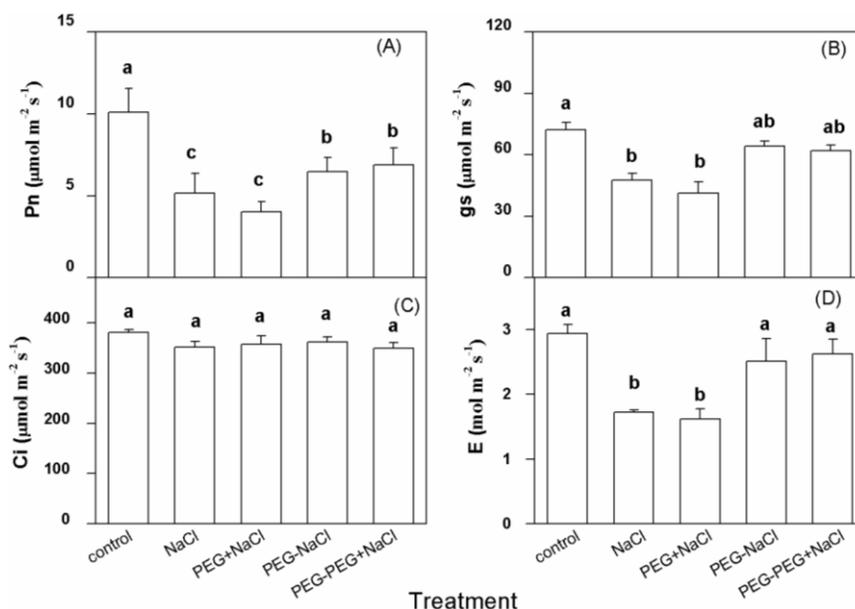


Fig. 2: Effects of 10% PEG pre-treatment on photosynthetic characteristics of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD ($n=3$). The different letters indicate significant differences at $p < 0.05$

Nevertheless, stomatal conductance (g_s) and transpiration (E) reduced significantly under NaCl or NaCl+PEG stress (Fig. 2b and 2d). The decrease in photosynthetic carbon assimilation may be due to the reduced stomatal conductance (g_s), the cumulative effects of other non-stomatal and biochemical components, and other factors (Brugnoli and Lauteri, 1991; Djanaguiraman *et al.*, 2006). Photosynthetic parameters in pre-treated plants were higher than of non-pretreated except internal CO_2 concentration (Fig. 2). The value of F_v/F_m significantly decreased under NaCl stress in chlorophyll fluorescence measurements (Fig. 3), is consistent with other studies of plants under salt stress (Lu *et al.*, 2003; Hao *et al.*, 2012). Our data also showed

that chlorophyll fluorescence parameter F_v/F_0 was the same sensitive to salt tolerant as F_v/F_m , and indicated by the same trends in the all treated plants. Interestingly, PEG pre-treatment increased the values of F_v/F_m and F_v/F_0 , effectively counteracted NaCl-initiated adverse influence on rice seedlings chlorophyll fluorescence.

Chlorophyll and carotenoid contents were also affected in non-pretreated plants. The chlorophyll contents significantly reduced in non-pretreated plants (Fig. 4a). According to Saha *et al.* (2010), the reason of reduction for chlorophyll contents under NaCl stress might be enhanced activity of chlorophyllase enzyme or due to the disruption of fine structure of chloroplast and instability of pigment

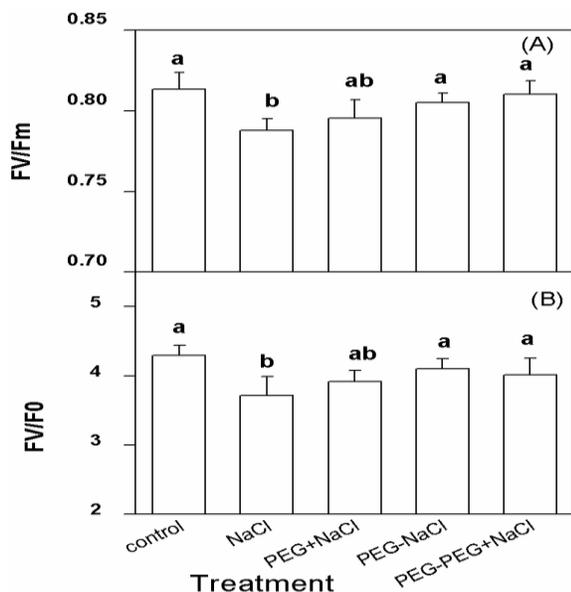


Fig. 3: Effects of 10% PEG pre-treatment on chlorophyll fluorescence of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD. The different letters indicate significant differences at $p < 0.05$

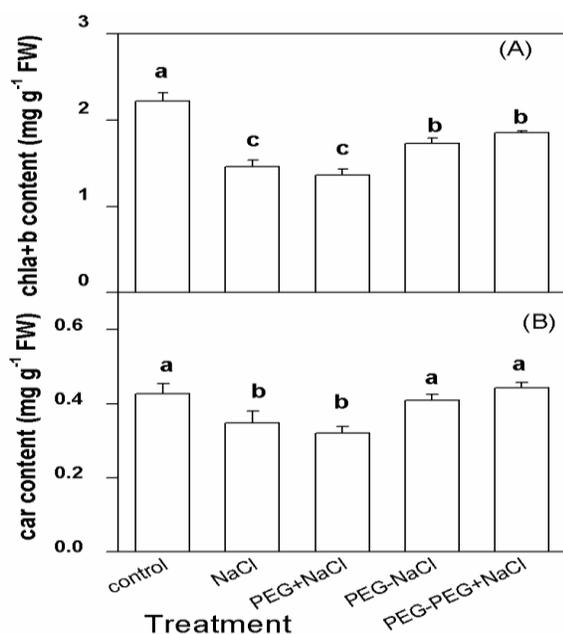


Fig. 4: Effects of 10% PEG pre-treatment on chlorophyll and carotenoid content of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD. The different letters indicate significant differences at $p < 0.05$

protein complexes by ions. The NaCl+PEG stress had significantly lower chlorophyll content than control plants (Fig. 4a). However, PEG pre-treatment had a beneficial effect on chlorophyll content in the NaCl or NaCl+PEG

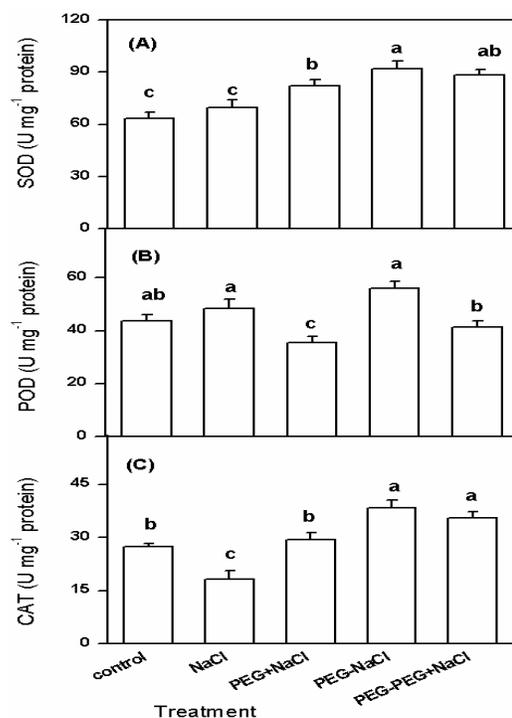


Fig. 5: Effects of 10% PEG pre-treatment on antioxidative enzyme activities of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD. The different letters indicate significant differences at $p < 0.05$

stress (Fig. 4a). Enhanced carotenoids may protect the thylakoid membrane in lipid phase.

Increasing evidence has demonstrated that NaCl and/or PEG resulted in an increase generation of reactive oxygen species (Neelma and Faheem, 2009; Shafi *et al.*, 2009). Plants adopt some defense mechanisms to protect themselves from the damaging effect of NaCl and/or PEG induced oxidative stress. SOD and POD activities were increased, whereas of CAT activities decreased under salt stress (Fig. 5). Hao *et al.* (2012) also reported the similar results that under salt stress damage led to significantly high SOD and POD activities and more lipid peroxidation to the lower CAT activity. However, Ma *et al.* (2012) found that pronounced increase in CAT activity under NaCl stress may be partly due to variation among species, developmental phase, stress intensity, and exposure time. The NaCl+PEG stress caused marked increase of SOD and CAT activities for rice plants, and significant decrease in POD activity (Fig. 5). Maybe, NaCl+PEG stress showed synergistic effects on oxidative stress. According to Guo *et al.* (2012), combined Cd and elevated O₃ had a markedly synergistic effect on oxidative stress for wheat shoots. However, Welfare *et al.* (2002) observed that NaCl and ozone in combination had an antagonistic effect for two chickpea varieties. Therefore, it is worth doing to explore

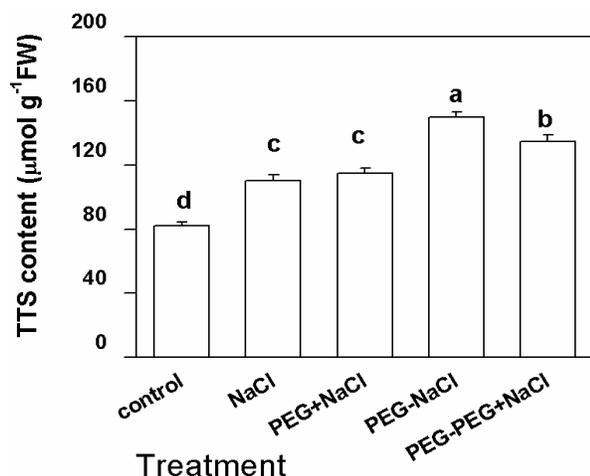


Fig. 6: Effects of 10% PEG pre-treatment on total soluble sugar content of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD. The different letters indicate significant differences at $p < 0.05$

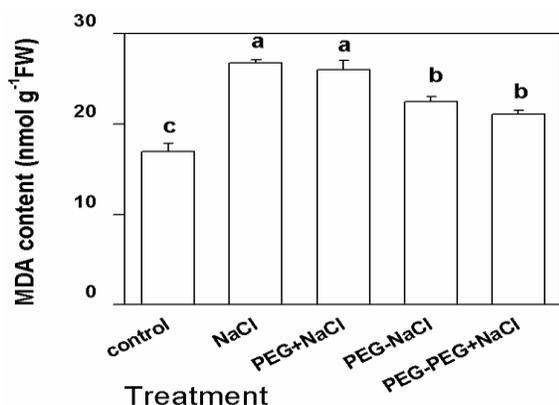


Fig. 7: Effects of 10%PEG pre-treatment on MDA content of rice seedlings subjected to NaCl or NaCl+PEG stress. Each value represents mean \pm SD. The different letters indicate significant differences at $p < 0.05$

the mechanisms about plants response to multiple stress factors in future. In PEG pre-exposed plants, the increase in the antioxidative enzyme activities was even more when compared with directly exposed to non-pretreated plants (Fig. 5). The increase of antioxidative enzyme activities might induce cross-adaptation on subsequent stress in PEG pre-exposed plants, which afford better survival conditions for plants exposed to subsequent NaCl or NaCl+PEG stress.

Besides antioxidant enzymes, soluble sugar are accumulated under different abiotic stress (Ashraf and Foolad, 2007; Garg and Chandel, 2011). The increased soluble sugar content was consistent with previous studies of Irigoyen *et al.* (1992) for water stress in nodulated alfalfa plants. There was higher soluble sugar content in plants exposed to PEG pre-treatment. This increase in the soluble

sugar content might be attributed to moderate PEG stress effective in osmoregulation to prevent excessive water loss and promoting resistance to subsequent NaCl or NaCl + PEG stress.

The current results showed that MDA content of NaCl or NaCl+PEG stress significantly increased in comparison with the controls, indicating the existence of oxidative stress (Fig. 7). These results corroborated previous studies, where NaCl+PEG stress caused severe lipid peroxidation (Neelma and Faheem, 2009). Increased MDA content might be due to presence of damaging ROS or inhibition of antioxidative defense systems. The present study also recorded that MDA content in PEG pre-treated plants decreased significantly (Fig. 7) showing that PEG pre-treatment alleviated the oxidative stress caused by NaCl or NaCl + PEG stress.

Conclusion

NaCl or NaCl+PEG stress reduced rice seedlings growth via complex mechanisms including the photosynthetic process, antioxidant pathway, and increase in soluble sugar content. PEG pre-treatment had positive effects in improving the plant growth and its capacity for tolerance to NaCl or NaCl+PEG stress through various physiological and biochemical pathway, as indicated increase in pigment content, net photosynthetic rate, stomatal conduction and transpiration, and antioxidative activities, etc. Cross-adaptation was induced by moderation of PEG to decrease subsequently the deleterious effects of NaCl or NaCl+PEG stress in rice seedlings. The obtained results suggest that plants may store the impression of previous stimuli to counter sequential stress. By PEG pre-treatment, plants may reach multiple tolerance for synchronously occurring salt and drought stresses in the field condition.

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