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Pretreatment with Spermidine Reverses Inhibitory Effects of Salt Stress in Two Rice (*Oryza sativa* L.) Cultivars Differing in Salinity Tolerance

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Abstract: Environmental stresses including soil salinity are severely affecting rice growth and productivity. The triamine Spermidine (Spd), a member of Polyamines (PAs), a group of phytohormone-like natural amine compounds has been shown to play essential roles in salt stress tolerance in many important crop plants. The effects of exogenously supplied Spd were investigated in two rice cultivars differing in salt tolerance level to determine the roles of Spd on the modulation of physiological parameters related to salt-stress responses. Thirty day-old seedlings of two rice cultivars, Pokkali (salt-tolerant) and KDML 105 (salt-sensitive), grown in nutrient solution were exposed or not to 1 mM Spd for 24 h before submitted to salinized solution containing 150 mM NaCl for 7 days. Salinity stress resulted in significant reduction in plant height, fresh and dry weight of both cultivars but Spd pretreatment significantly increased these growth parameters only in Pokkali. Salt-stress induced considerable disturbance in several physiological processes inhibitory for growth including chlorophyll loss, accumulation of hydrogen peroxide and increase in lipid peroxidation, increased electrolyte leakage and increase in Na^+/decrease in $K+/Na^+$. Spd pretreatment led to the reversal of those inhibitory effects in both cultivars. This study showed that exogenous Spd can be applied as short-term pretreatment prior to introduction of salt stress to help elevate salt tolerance of rice and confirmed earlier observations that exogenous Spd offered protective roles on salinity-stressed rice by stabilizing membrane, scavenging free radicals and maintaining K^+/Na^+ status.

Key words: Exogenous spermidine, hydrogen peroxide, malonyldialdehyde, *Oryza sativa* L., salinity tolerance

INTRODUCTION

Salinity is one of the most damaging abiotic stress factors limiting crop productivity worldwide. High concentration of salts in the soils immediately imposes on plants the osmotic stress effect due to lower soil water potential leading to retardation of water uptake. When exposed for longer period, salinity entails ionic stress when plants absorb and accumulate toxic level of Na^+ and Cl^- in the cytoplasm. Salinity also induced secondary stresses such as nutritional imbalance and oxidative stress (Zhu, 2002). Plants have to evolve many adaptive strategies primarily to protect against water loss and ion toxicity including synthesis of compatible solutes, exclusion and compartmentalization of salts at the cellular level and control of ion uptake by roots and transport into shoots. Furthermore, plants have to employ a wide range of biochemical and molecular mechanisms including alteration of photosynthetic pathway, change in membrane structure, stimulation of phytohormones and

induction of antioxidative enzymes (Parida and Das, 2005; Mudgal *et al.*, 2010). Polyamine (PA) is a group of low-molecular-weight aliphatic amines which is ubiquitous in plant cells. PAs are essential for plants and postulated to be involved in a wide range of biological processes including, cell division, growth and development, senescence and the responses of plants to biotic and abiotic stresses (Galston and Kaur-Sawhney, 1990). The major form of PAs in plants includes Putrescine (Put), Spermidine (Spd) and Spermine (Spm). The diamine Put serves as the obligate precursor of Spd and Spm which are formed by subsequent addition of an aminopropyl moiety onto Put and Sdp, respectively (Bouchereau *et al.*, 1999). All three forms of PAs have been shown to accumulate under biotic and abiotic stresses and implicated to play essential roles in stress tolerance of plants. Changes in endogenous PAs under stress may be affected by several factors including plant species or cultivars, duration and intensity of stress treatment, developmental stage of tissues, hormonal application and

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types of fertilizer (El-Khawas, 2004; Liu *et al.*, 2007; Rapatsa and Terapongtanakorn, 2010). The physiological mechanism of PAs in plant stress is not thoroughly understood. Due to their polycationic nature at physiological pH, PAs have been reported to be involved in stabilization of biological membrane and macromolecular structures of cells by directly binding to anionic macromolecules including membrane phospholipids, nucleic acids and proteins (Alcazar *et al.*, 2006). Gene expression analysis of transgenic plants overexpressing polyamine biosynthesis genes revealed changes in expression of several stress-related genes, suggesting that polyamines may also play a role as signaling molecules in plant response to stress (Capell and Bassie, 2005; Wi *et al.*, 2006).

Exogenous PA application has been successfully used to enhance stress tolerance including salinity tolerance of plants (Anjum *et al.*, 1999; Chattopadhyay *et al.*, 2002; Iqbal and Ashraf, 2005; Verma and Mishra, 2005; Duan *et al.*, 2008; Roychoudhury *et al.*, 2011). Exogenous PAs have been shown to modulate levels of endogenous PAs and a large body of evidence suggests that exogenous PAs could reverse growth inhibition caused by stresses. Beneficial effect on growth was attributed to the mitigation of stress-derived inhibitory effects including cell membrane damage, Reactive Oxygen Species (ROS) accumulation and accumulation of Na^+ and Cl^- ions in different organs. Exogenous PAs also enhanced the expression of osmotically responsive genes and activities of antioxidant enzymes (Hussain *et al.*, 2011).

In rice, the protective roles of exogenous PAs has been attributed to the reduction of salinity stress-induced damages. These included an accumulation of Na^+, loss of K^+, loss of chlorophyll, inhibition of photochemical reaction of photosynthesis, down-regulation of chloroplast-encoded photosynthetic genes, ROS accumulation, lipid peroxidation and membrane damage. Exogenous PAs were also reported to enhance the activity of antioxidative enzymes and increase synthesis of non-enzymatic antioxidants and compatible osmolytes (Chattopadhyay *et al.*, 2002; Roychoudhury *et al.*, 2011). Salt stress increased endogenous Put, Spd and Spm in varying degrees among different rice cultivars (Krishnamurthy and Bhagwat, 1989; Lefevre *et al.*, 2001; Roychoudhury *et al.*, 2011) and exogenous PAs in combination of NaCl either had no effects or further increase endogenous PAs, particularly Spd and Spm (Roychoudhury *et al.*, 2011). Some authors, however, reported a salinity-induced decrease in Put, Spd and Spm (Lin and Kao, 2002). These authors also found that in salt-tolerant rice cultivars, NaCl stress and exogenous PAs resulted in more pronounced elevation of Spd/Spm than

Put and related high Spd/Spm and low Put with salt tolerance ability. Ndayiragijie and Lutts (2007) demonstrated that Put and to a lesser extent Spd, reduced the deleterious impact of salinity on yield of a salt-sensitive rice variety while Spm had no positive effect. In contrast to most reports, Ndayiragije and Lutts (2006) reported that addition of PAs in culture solution did not improve growth of seedlings of a salt-sensitive rice and induced a strong increase in lipid peroxidation and cell membrane stability. Therefore, it seems that the impact of PAs in salt stress resistance remains disputed. The underlying physiological significance of PAs in stress response is complicated by the fact that distinct PAs may assume distinct functions in different plant species or among varieties of the same species (Roychoudhury *et al.*, 2011).

This study was undertaken to investigate the effects of exogenous Spd on salt-induced reduction in growth and modulation in physiological parameters related to salt tolerance in two rice cultivars differing in salinity tolerance. Previous studies in rice involved the application of PAs simultaneously with the salt stress treatment, this study will also investigate whether Spd pretreatment prior to salt application will also enhance the ability of rice seedlings to resist salt stress.

MATERIALS AND METHODS

Plant materials and treatments: Two cultivars of rice (*Oryza sativa* L.), Pokkali (salt-tolerant) and KDML105 (salt-sensitive) were used. The experiment was performed in a greenhouse under natural photoperiod at the Faculty of Agriculture, Khon Kaen University during the months of May to July, 2009. Seeds were surfaced-sterilized, soaked in sterile distilled water for 48 h and germinated in Petri plates lined with two layers of paper towel moistened with sterile distilled water. Seven-day-old seedlings of uniform size with three leaves were transplanted to plastic containers holding 20 L of full-strength Yoshida nutrient solution pH 5.0 (50 seedlings per container) (Yoshida *et al.*, 1976). The solution was renewed weekly and the pH was adjusted daily. On day 24, two treatment groups were pretreated with Spd by adding 1 mM Spd into nutrient solution. After 24 h of Spd pre-treatment, plants of one treatment group (Spd) were transferred to normal nutrient solution whereas those of the other to nutrient solution containing 150 mM NaCl (NaCl+Spd). On the same day, plants of another treatment group were transferred from normal nutrient solution to the one containing 150 mM NaCl (NaCl). One set of plants cultured in normal nutrient solution throughout the experiment served as the control. After 7 days of salt

treatment, plants were collected for determination of growth and physiological changes. The experiment was arranged in a completely randomized design with five replicates.

Determination of growth: To determine the growth, shoot length and fresh weight were measured from ten plants randomly selected from five replicated containers. Plant dry weight was determined by drying in a 65°C hot air oven for one week or until the weight became constant.

Determination of membrane damage, hydrogen peroxide and lipid peroxidation: Freshly harvested leaf samples (0.1 g) were washed thoroughly with deionized water, blotted and then transferred to a tube containing 10 mL of deionized water and incubated at 32°C for 2 h. The Electrical Conductivity (EC1) of the leachates was measured and then the tissues were killed by autoclaving at 0.1 Mpa for 20 min to release all electrolytes. The final Electrical Conductivity (EC2) was measured and the level of membrane injury was expressed as percentage of initial EC based on the final EC according to Dionisio-Sese and Tobita (1998). Hydrogen peroxide (H_2O_2) content was determined according to Velikova *et al.* (2000). Leaf tissues were extracted on ice with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). After the cell debris was removed by centrifugation, the supernatant was added with 0.5 mL of 10 mM potassium phosphate buffer and 1 mL of 1 M Potassium iodide. The absorbance of mixture was read at 390 nm and the content of H_2O_2 was calculated from a standard curve. The level of lipid peroxidation was represented by malondialdehyde (MDA) content which was measured according to Harding and Benson (1995). Leaf samples were added with 1.4 mL distilled water and 1.5 mL of Thiobarbituric Acid (TBA) reagent. The mixture was boiled in water bath for 25 min and the reaction was stopped by placing the tube on ice. Then, the absorbance of supernatant was read at 532 and 600 nm. The MDA concentration was determined by its extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of Na$^+$ and K$^+$: To quantify the Na$^+$ and K$^+$ concentrations in shoots, tissues were oven-dried at 70°C for 72 h. The 0.1 g dry powder samples were heated in a muffle furnace at 500-600°C and the ashes were dissolved in 0.25 M HCl. Ion determination were made by atomic absorption spectrometer (Corning, Model GBC 932 AAA, England) and the concentrations were calculated from a standard curve for each ion.

Determination of free proline and total chlorophyll: Total chlorophyll content was estimated from 80% (v/v) acetone extraction. The absorbance of the extracts was measured at 645 and 663 nm. Chlorophyll content was calculated from the absorbance readings according to Arnon (1949). The free proline was quantified from the sulphosalicylic acid extracts which was reacted with ninhydrin reagent, boiled for 60 min and extracted with toluene. The absorbance of the colored product in the toluene phase was read at 520 nm. The proline content was determined from a standard curve and calculated on a fresh weight basis as described by Bates *et al.* (1973).

Statistical analysis: Statistical evaluation concerning all parameters was analyzed as CRD with five replications using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL). All data were analyzed by the Analysis of Variance (ANOVA). Significant difference at $p < 0.05$ among treatments were determined by Duncan's Multiple Range Test (DMRT).

RESULTS

Salinity treatment using 150 mM NaCl for 7 days obviously resulted in an overall growth retardation of both rice cultivars compared to the controls as clearly shown in Fig. 1, which showed that lower leaves of salt-
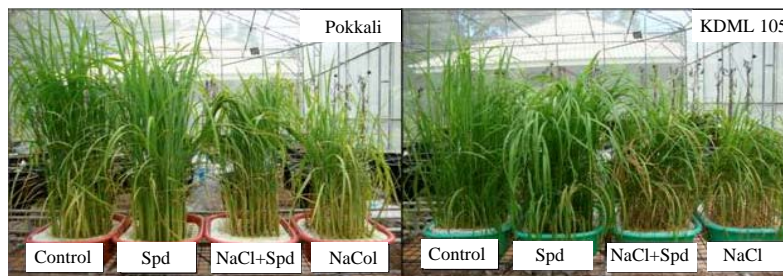

Fig. 1: Effect of Spd pre-treatment on growth of 37 day old plants of rice cvs. Pokkali and KDML 105 subjected to salt-stress by 150 mM-NaCl treatment for 7 days
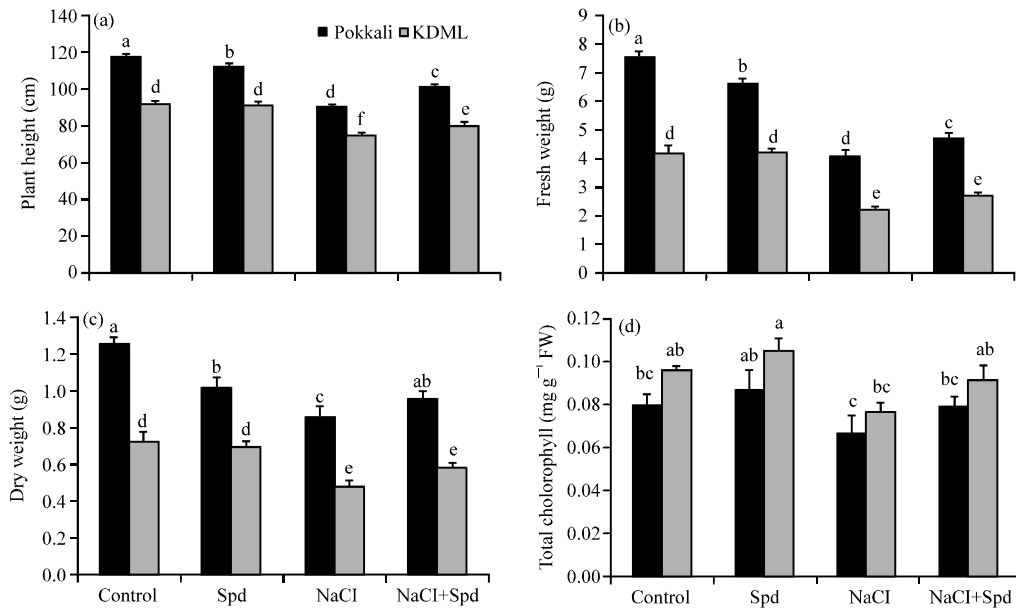
Fig. 2(a-d): Effects of exogenous Spd (1 mM) pretreatment on height (a), fresh weight (b) dry weight (c) and total chlorophyll content of rice plants stressed with 150 mM NaCl for 7 days. Each histogram in a, b and c represents a mean value of ten and d for five replicated measurements and the bars indicate SE. Mean values with different letters are significantly different at p<0.05

stressed plants, especially KDML 105 rolled and completely dried out. While leaves of the control plants remained green and healthy, the younger leaves of stressed plants were light green, some were partly yellowish and showed symptoms of leaf rolling and drying near the tips. Such leaf symptoms were more pronounced in KDML 105 than Pokkali. It also appeared that salt-stressed plants of Pokkali remained upright whereas those of KDML 105 were weaker and tended to bend down. Salt-stressed symptoms were somewhat alleviated by Spd pretreatment. Plant height of both cultivars was significantly reduced under salt stress showing 23 and 19% reduction for Pokkali and KDML 105, respectively. Spd pretreatment increased plant height under salt stress (Fig. 2a). Spd caused an increase in plant height of 11.9% for Pokkali and 6.4% for KDML 105. Plant fresh and dry weight significantly decreased as a result of salinity. The percentage reduction in fresh weight was 46% in both cultivars and the reduction in dry weight was 31.7% in Pokkali and 33% in KDML105 (Fig. 2b, c). When comparing fresh and dry weight of NaCl-stressed plants with NaCl-stressed plants pretreated with Spd, it was found that Spd resulted in an increase in plant fresh weight by 15.5% in Pokkali and 22.7% in KDML 105 and dry weight increase was 11.6% in Pokkali and 20.4% in KDML 105. In Pokkali, the reduction in plant height, fresh and dry weight due to salt-stress was significantly improved by Spd pretreatment, whereas for KDML 105 only plant height was significantly improved. Loss of

chlorophyll due to salt stress was 16.6 and 20.1% in Pokkali and KDML 105, respectively (Fig. 2d). The reversion of salinity-induced loss of chlorophyll was observed in both cultivars, although the mean values between the NaCl and the NaCl+Spd treatments were not statistically different.

As shown in Fig. 3a, the level of lipid peroxidation as assayed by the concentration of MDA increased slightly under salt stress (14.2% for Pokkali and 8.3% for KDML 105). Spd pretreatment effectively lowered the level of lipid peroxidation in NaCl-stressed plants i.e., 23% reduction in both cultivars. Spd also tended to reduce lipid peroxidation in non-stressed plants. Salinity stress induced the accumulation of H_2O_2 and Spd pretreatment significantly reversed that effect in both cultivars bringing H_2O_2 level down to that comparable to the controls (Fig. 3b). Similar to lipid peroxidation, Spd tended to be beneficial for non-stressed plants by slightly lowering the concentration of H_2O_2. Salinity was highly deleterious to membrane structure causing a dramatic increase in electrolyte leakage (3.9 times in Pokkali and 5.75 times in KDML 105) as shown in Fig. 3c. In the absence of NaCl, Spd had no effect on modulation of membrane structure. Spd pretreatment apparently helped reduce the salt-induced membrane damage, especially in KDML 105 (28.2% reduction compared to 14.3% reduction for Pokkali). Proline concentrations enormously increased by 2.4-folds in Pokkali and 7.2-folds in KDML 105 with NaCl stress (Fig. 3d). Spd treatment caused a substantial and
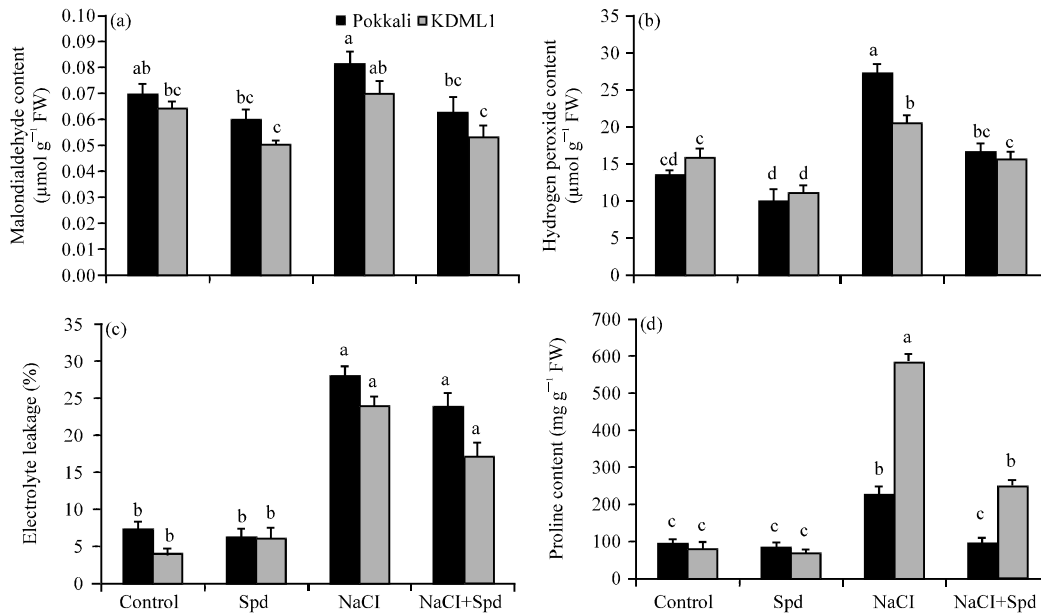
Fig. 3(a-d): Effects of exogenous Spd (1 mM) pretreatment on MDA (a), hydrogen peroxide (b), electrolyte leakage (c) and proline (d) in leaves of 37 day old rice subjected to 150 mM NaCl treatment for 7 days. Each histogram represents a mean value of five replicated measurements and the bars indicate SE (n = 5). Mean values with same letters are not significantly different
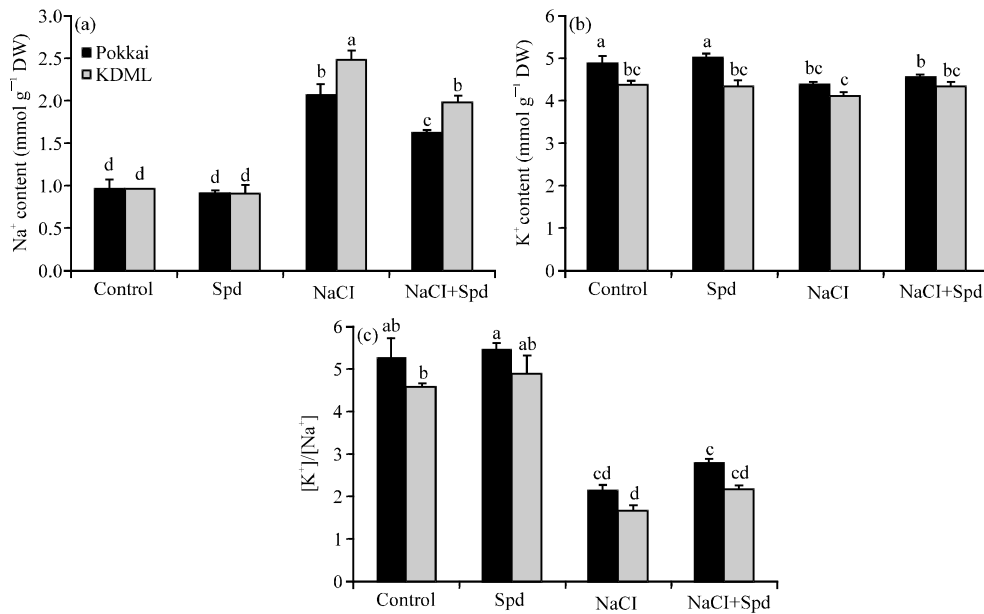

Fig. 4(a-c): Effects of exogenous Spd (1 mM) pretreatment on Na^+ (a) and K^+ (b) concentrations and K^+/Na^+ ratio in leaves of 37 day old rice subjected to 150 mM NaCl treatment for 7 days. Each histogram represents a mean value of five replicated measurements and the bars indicate SE (n = 5). Mean values with same letters are not significantly different

comparable reduction in proline (about 57-58%) in both cultivars. Spd, however, did not alter proline metabolism of non-stressed plants.

As shown in Fig. 4a, salinity stress resulted in large and significant increase in Na^+ concentration in the leaves of both cultivars (an increase of 53.4% in Pokkali and

61.3% in KDML 105) and Spd application led to a significant decrease. On the contrary, salinity caused a reduction in K^+ but exogenous Spd treatment did not alter K^+ concentrations (Fig. 4b). Corresponding to the changes in Na^+ and K^+ contents, the ratio of $[K^+]/[Na^+]$ was considerably decreased by salt stress (from 5.23 to 2.15 in Pokkali and 4.59 to 1.69 in KDML 105). Spd pretreatment led to a slight non-significant increase in $[K^+]/[Na^+]$ in both cultivars (Fig. 4c). In the absence of NaCl stress, Spd did not have any impact on K^+-Na^+ homeostasis of rice plants.

DISCUSSION

Exogenously applied PAs have been shown to be beneficial in sustaining growth and reducing deleterious effects of salt stress in several plants including mulberry (Das *et al.*, 2002), bean (Zeid, 2004), *Cucumis sativus* (Duan *et al.*, 2008) and canola (Sakr and Arafa, 2009). In rice, beneficial effects of exogenous PAs, applied in combination with NaCl, in relieving salt-stress effects and promoting growth have been demonstrated when applied at the seedling stages (Chattopadhyay *et al.*, 2002; Roy *et al.*, 2005; Roychoudhury *et al.*, 2011). Moreover, the modulation by PAs affected salt-tolerant and salt-sensitive rice cultivars to different extents. The results in this study also revealed that an exogenous PA (Spd) had a protective role in counteracting salt stress effects leading to improved growth-related parameters particularly for the salt-tolerant cultivar, Pokkali (Fig. 2a-c). In contrast, an earlier report (Roychoudhury *et al.*, 2011) demonstrated that exogenous PAs had more profound effects on growth of salt-sensitive (M-1-48 and Gobidobhog) than salt-tolerant rice (Nonabokra). This incongruence may be explained mainly by the use of different cultivars and plant age, growing conditions and level and duration of salt stress.

The protective roles of Spd were ascribed to reduction of chlorophyll loss (Fig. 2D); lipid peroxidation, membrane damage and hydrogen peroxide (Fig. 3) and improvement of K^+/Na^+ homeostasis (Fig. 4). Protective roles of exogenous PAs (Spd and Spm) in chlorophyll loss were previously reported in rice (Chattopadhyay *et al.*, 2002; Roychoudhury *et al.*, 2011). Salt-induced loss of chlorophyll was accompanied by inhibition of photochemical reactions and down-regulation of chloroplast-encoded genes, all of these inhibitory effects could be reversed by exogenous Spd and Spm (Chattopadhyay *et al.*, 2002). Tassoni *et al.* (2000) reported that *Arabidopsis thaliana* seedlings absorbed exogenous Spd from the growth medium and distributed it to leaves and resulted in an increased amount of total chlorophyll and delaying leaf senescence. The evidence that PAs were absorbed, metabolized and bound with photosynthetic proteins in chloroplasts suggested its promising role in stabilizing oligomeric photosynthetic proteins particularly the chl a/b binding proteins from protease action during stress (Del Duca *et al.*, 1995).

In line with previous reports, this study demonstrated that salinity induced generation of excessive amount of ROS such as H_2O_2 (Fig. 3b) and increased H_2O_2 in turn led to lipid peroxidation (Fig. 3a) causing membrane damage and leakage of electrolytes (Fig. 3d). These physiological changes were correlated with overall growth reduction and exogenous Spd effectively alleviated those adverse physiological effects and resulted in some growth improvement, particularly for Pokkali. Protective roles of PAs in these respects have been reported in rice (Chattopadhyay *et al.*, 2002; Roychoudhury *et al.*, 2011), *Brassica juncea* (Verma and Mishra, 2005) and *Cucumis sativa* (Duan *et al.*, 2008). Due to their polycationic nature, PAs were suggested to bind directly to the negatively charged membrane phospholipid headgroups thereby help in stabilizing the membrane and positively influencing membrane stability (Schuber, 1989). An ability of plants to scavenge free radicals by inducing antioxidative systems under salt stress is an important component of stress protective mechanism. Exogenous PAs protected stressed plants by further enhancing the activity of antioxidant enzymes including catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase, as well as increase the production of non-enzymatic antioxidants including anthocyanin, carotenoids and reduced glutathione (Verma and Mishra, 2005; Duan *et al.*, 2008; Roychoudhury *et al.*, 2011). Using the transgenic approach, He *et al.* (2008) demonstrated that increase of Spd content in transgenic lines of pear overexpressing *Spermidine synthase* gene led to enhancement of enzymatic and non-enzymatic antioxidant capacity of NaCl and mannitol-stressed plants resulting in better growth and less injury compared with the stressful non-transformants.

Exogenous Spd alleviated salt-induced damage by reducing the accumulation of Na^+ and preventing loss of K^+ leading to an improvement of K^+/Na^+ in rice leaves (Fig. 4). This beneficial effect of Spd under salt stress has been previously demonstrated in rice (Chattopadhyay *et al.*, 2002; Roy *et al.*, 2005; Ndayiragije and Lutts, 2006; Roychoudhury *et al.*, 2011). Zhao *et al.* (2007) also found in barley seedlings that the shoot K^+/Na^+ was increased significantly by exogenous Spd which acted by regulating ion channel activities in root cells to repress Na^+ influx into roots and increase K^+

influx from roots to shoots. Moreover, Roy *et al.* (2005) found that the activity of plasma membrane-bound H^+-ATPase in rice roots, which was inhibited by salt stress, was dramatically stimulated by exogenous Spd contributing to reduction in Na^+ and increase in K^+ in salt-stressed roots. X-ray microanalysis imaging of barley roots revealed that exogenous Spd inhibited Na^+ transport from exodermis to xylem vessel by strengthening the barrier effects of Casparian bands suggesting the multi-faceted nature of Spd action (Zhu *et al.*, 2006). Therefore, it appeared that exogenous Spd help relieve plants from damaging effects of salt stress by reversing inhibitory effects on several crucial biochemical and physiological mechanisms contributing to salt tolerance.

Proline accumulation is another physiological parameter believed to play adaptive roles in plant abiotic stress tolerance including salinity stress (Verbruggen and Hermans, 2008). Proline has been proposed to act as a compatible osmolyte and storage materials for nitrogen during stress recovery (Hare and Cress, 1997), a ROS scavenger (Smirnoff and Combes, 1989) and a modulator of $NADP^+$/NADPH redox state (Phang, 1985). Rice cultivars accumulating higher proline under salt stress were able to maintain higher green leaf percentage (Pongprayoon *et al.*, 2008). However, in some species or ecotypes, the data do not always indicate a positive correlation between proline accumulation and adaptation to drought and salt stress (Lutts *et al.*, 1999; Hien *et al.*, 2003; Theerakulpisut *et al.*, 2005). Some authors even suggested that salt-induced proline accumulation is often a late response, appearing only when cell injury is evident (Chandler and Thorpe, 1987; Lutts *et al.*, 1996). Salt-induced proline accumulation in both rice cultivars was suppressed by Spd pretreatment and in Pokkali the proline level returned to that comparable to the control (Fig. 3d). This action of exogenous Spd in reducing the amount of salt-induced proline, in association with improved leaf yield, chlorophyll and photosynthetic efficiency, was as also reported by Das *et al.* (2002) in mulberry. In contrast, Roychoudhury *et al.* (2011) found that exogenous Spd further enhanced proline accumulation in all three rice cultivars differing in salt tolerance. Furthermore, Duan *et al.* (2008) reported similar effect of Spd in cucumber. The opposing effects of exogenous Spd on proline accumulation may be explained partly by varying experimental systems used in different studies including different plant species or cultivars of the same species, growth stages, tissue types, concentrations of NaCl and duration of stress and concentrations and modes of application of Spd. The polyamine synthesizing and oxidizing pathways are intricately connected to proline synthesis and degradation pathways and these pathways

use glutamate, ornithine and arginine as common precursors (Mohapatra *et al.*, 2010). Exogenously supplied Spd which has been shown to perturb the metabolism of PAs by altering endogenous levels of Put, Spd and Spm under salt stress (Krishnamurthy and Bhagwat, 1989; Ndayiragije and Lutts, 2006; Roychoudhury *et al.*, 2011) is expected to have a profound effect on substrate availability for the interconnected pathways including those involved in synthesis and degradation of proline.

Although, beneficial effects of exogenous Spd on mitigating the adverse effects of salt stress on physiological processes leading to enhanced tolerance and better growth performance have been well-documented, the mechanisms of action of Spd and other PAs, at the molecular levels are not yet fully understood. Further studies using modern biological disciplines like electrophysiology, microarray, transcriptomics, proteomics and metabolomics will be helpful to improve our understanding of the involvement of PAs in biotic and abiotic stress tolerance (Hussain *et al.*, 2011). Genetic manipulation of polyamine metabolism (Roy and Wu, 2001, 2002; Kumria and Rajam, 2002; Capell *et al.*, 2004; He *et al.*, 2008; Perimarti *et al.*, 2009) not only serves as a valuable tool for studying physiological roles of PAs in modulating salinity and drought stress responses but also provides plant breeders with a promising approach for biotechnological improvement of crop salinity and drought tolerance. Nonetheless, the use of exogenously applied PAs to improve salt tolerance of rice is a simple and interesting alternative.

CONCLUSION

Salt stress caused a significant growth reduction during the vegetative stage of both salt-tolerant and salt-sensitive rice cultivars. Application of Spd prior to stress treatment confers better tolerance to salt stress, particularly in the salt-tolerant cultivar. Salt-induced damages including chlorophyll loss, membrane leakage, ROS (H_2O_2) accumulation, lipid peroxidation, Na^+ accumulation and reduction in K^+ were all relieved in Spd-pretreated plants resulting in improved growth performance. An experiment employing Spd pretreatment at the reproductive stage is being investigated to study its effects on enhancement of salt tolerance and yield improvement of rice.

ACKNOWLEDGMENT

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