Effects of salinity on photosynthesis and growth of rice and alleviation of salt-stress by exogenous spermidine

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ABSTRACT: This research aimed to study photosynthesis and growth of rice under salt-stress conditions and the efficiency of spermidine (Spd) to alleviate salt-stress effects during the vegetative stage. Two rice cultivars including Pokkali (salt-tolerant) and KhaoDawk Mali 105 (KDML105; salt-sensitive) were used. Rice were grown in pots until 28 days old, the plants were then divided into four groups consisting of one control(without NaCl and Spd)) and three treatments including (1) Spd (2) NaCl and (3) NaCl + Spd. For the Spd and NaCl + Spd group, the plants were treated with 0.5 mMSpd as a foliar spray for 7 successive days. After that salinity treatment was imposed to NaCl and NaCl + Spd treatments by adding 150 mMNaCl to the pots for 14 days. The results showed that salinity stress inhibited growth of KDML105 more than Pokkali and Spd treatment could improve dry weight and tilleringin salt-stressed KDML105. Salinity significantly reduced stomatal conductance, transpiration and membrane integrity but had minimal effects on net photosynthesis rate and maximum efficiency of photosystem II (PSII). Spd treatment could alleviate salt-stress effects in KDML105mainly by protecting membrane integrity and enhancing PSII functions.

Keywords: photosynthesis, rice, salt-stress, spermidine

Introduction

Nowadays, 50% of the world's irrigated land area is adversely affected by salinity (Hu and Schmidhalter, 2005). Salinity is a stressful condition that causes a significant reduction in plant production worldwide. Yield of all major crops are primarily determined by photosynthesis (Long et al., 2006). Primary effects of salinity on photosynthesis involve an induction of stomatal closure which limits of CO_2 diffusion and assimilation. Salinity can also cause secondary effects such as oxidative stress (Chaves et al., 2009). Limitation of CO_2 fixation causes imbalance of light capture and light utilization which generates excess amount of toxic reactive oxygen species (ROS) (Miller et al., 2010). Moreover, uptake and accumulation of salts also leads to ion toxicity effects which modulate various important proteins relating to photosynthetic functions (Silveira and Carvalho, 2016; Wani et al., 2013).

Rice (*Oryza sativa* L.) is a main food crop and is the staple food of more than half of the world's population (Saikrishna et al., 2018). For Thailand Thai jasmine rice, KhaoDawk Mali 105 (KDML105) is known as the best quality rice (Lanceras, et al., 2000). Salinity stress is one of the most constraining factors influencing production for KDML105 in some areas in northeast Thailand (Chutimanukul et al., 2018). Treating plants with plant growth regulators (PGR) was reported as one possible strategy to alleviate the effects of salinity on rice. Polyamine (PA) is a class of PGR previously reported to have protective roles on salt-treated rice (Bimpong et al., 2016; Saleethong et al., 2016). At physiological concentrations polyamines (PAs) for example spermidine (Spd) and spermine (Spm) significantly prevented the leakage of electrolytes

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and amino acids from roots and shoots induced by salinity stress (Chattopadhayay et al., 2002).

This research investigated the effects of salinity on photosynthesis and growth of rice under salt-stress conditions, as well asthe efficiency of Spd to alleviate salt-stress during the vegetative stage of rice.

Materials and Methods

Plant materials and treatments

Two rice cultivars including KDML105 and Pokkaliwere used. Each treatment had three replications each containing two plants.This experiment was designed by using completely randomized design.The seeds were obtained from Rice Gene Discovery Unit (RGDU), KasetsartUnivesity,Kamphaengsaen.Seeds were surface - sterilized with 15% sodium hypochlorite for 15 minutes, then rinsed with distilled water 5 times. The seeds were germinated in dark conditions for 2 days. Seedlings were transferred to plastic pots containing 3.5 kg of drypaddy soils. After 14 days from germination, the water level in each pot was maintained at 5 cm above the soil surface using tap water. When the plants were 28 days old, the plants were divided into four groups consisting of one control(without NaCl and Spd) and three treatments including (1) Spd, (2) NaCl and (3) NaCl + Spd. For the Spd and NaCl + Spd groups, the plants were treated with 0.5 mMSpd as a foliar spray at a volume of 20 mL per pot for 7 successivedays. The same amount of distilled water was sprayed in the control and the NaCl treatment groups. After the Spd spraying ended, salinity treatment was imposed to NaCl and NaCl + Spd groups by adding 150 mMNaCl to the pots, while the control was continued to be fed with tap water. The plants were harvested to measure physiological parameters after treated with NaCl for 14 days.

Measurement of photosynthesis

Photosynthesis was measured by using *LI-COR 6400XT* portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) using the following conditions; light intensity at 1,500 μmol(photon) m^{-2} s^{-1}, chamber temperature at 30 °C, andCO_2 concentration at 400 μl l^{-1}. The photosynthetic parameters recorded include net photosynthetic rate,stomatal conductance, transpiration rate and chlorophyll fluorescence (maximum PSII efficiency; Fv´/Fm´).

Measurement of electrolyte leakage(EL)

Leaf samples were cut into pieces 1 cm long. Six pieces were placed in a test tube containing 10 ml of deionized distilled waterandkept in the dark for 24 hours. The samples were measuredelectrical conductivity (EC) and recorded as EC1. The test tube was then boiled at 100 °C for 20 minutes to release all electrolytes, and then cooled to room temperature, the EC was measured again and recorded as EC2. Electrolyte leakage was calculated using the equation: $EL = EC1/EC2 \times 100$

Measurement of plant growth

After the plants were treated with NaCl for 14 days, plant growth parameters were measured including shoot dry weight (SDW) and root dry weight (RDW). For dry weight measurement, all samples were dried in hot air oven at 70 °C for one week.

Statistical analysis

All data was analyzed using analysis of variance to compare means among treatments. In any cases, $p<0.05$ is considered significant. All data analyses were performed using Statistix 10 data analysis software.

Results

As shown in Figure 1, growth ofKDML105 stressed with NaCl for 14 days (S) was clearly inhibited. The stressed plants (S) haveshorter and smaller root mass, lower shoot length and more dead leaves than the control (C). Formation of tillers was also inhibitedhaving only one tiller compared with two in the control plant. Salt-stressed plants pretreated with Spd (Spd+S) clearly showed better growth than stressed plants without Spd (S) i.e. thicker root mass, higher shoot length, more tillers and higher number of leaves which remained green. For Pokkali, growth of salt-stressed plants was slightly inhibited, and growth-promoting effects of Spd was more apparent for roots than shoots.

Rice plants of both cultivars treated with NaCl for 14 days showed a slight reduction in net photosynthesis rate compared to the controls (7.85 and 4.12% reduction for KDML105 and Pokkali, respectively) although not significantly different (**Figure 2A and B**).Stomatal conductance and transpiration rate, however, decreasedsignificantly under salinity stress i.e. 55 and 59% reduction for KDML105 and Pokkali, respectively (**Figure 2C, D, E and F**).Under salinity stress, the maximum PSII efficiency (Fv´/Fm´) slightly decreased in KDML105 (3.33% reduction) but increased in Pokkali (33.96% increase), although not significantly different from control (**Figure 2G and H**). Pretreatment with Spd slightly increased net photosynthesis rate only in Pokkali for both non-stressed (14.37% increase) and salt-stressed (13.37%) plants (**Figure 2B**). For both cultivars, Spdtreatment had no effects on stomatal conductance and transpiration rate of salt-stressed plants but slightly increased these parameters in non-stressed plants (**Figure 2C, D, E and F**).Under salt stress, the maximum PSII efficiency (Fv´/Fm´) slightly reduced (3.33%) for KDML105 but showed a 33.96% increase for Pokkali. However, Spd pretreatment tended to have a beneficial effect on PSII function of KDML105 (**Figure 2G**) but not Pokkali (**Figure 2H**). Salinity stress damaged plant membranes as indicated by significant increase in electrolyte leakage, and KDML105 was more damaged than Pokkali (**Figure 2I and J**). Electrolyte leakage from leaves of KDML105 increased significantly from the mean value of 10.8% in the control to 20.6% in the stressed plants (i.e. approximately 90% increase) while the mean values for Pokkali increased slightly from 5.9% to 8.4% (non-significant).

Pretreatment with Spdeffectively protected KDML105 from membrane damage in both non-stressed (reduced from 10.8% to 6.8%) and salt-stressed (reduced from 20.6% to 15.8%) plants. However, Spd tended to exacerbate the effects of salt stress causing higher electrolyte leakage in the Spd+S than the S plants. For KDML105, salinity stress was inhibitory, on dry weight basis, to root but not to shoot growth (which increased slightly), and Spd treatment had a tendency to be beneficial in promoting growth of stressed plants (**Figure 3A and C**). For Pokkali, salinity stress had a slight negative effects on root growth but promoted shoot growth. In contrast to KDML105, Spd treatment did not have any beneficial effects on growth of Pokkali (**Figure 3B and D**).

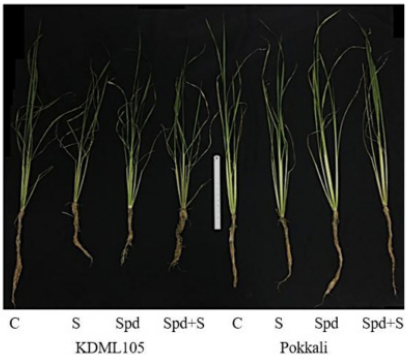

Figure 1 Forty-nine-day-old rice plants 14 days after salinity stress; C, control non-stressed plants; S, salt-stressed plants without Spdpretreatment;Spd, non-stressed plants with Spdpretreatmen; Spd+S, salt-stressed plants with Spd pretreatment

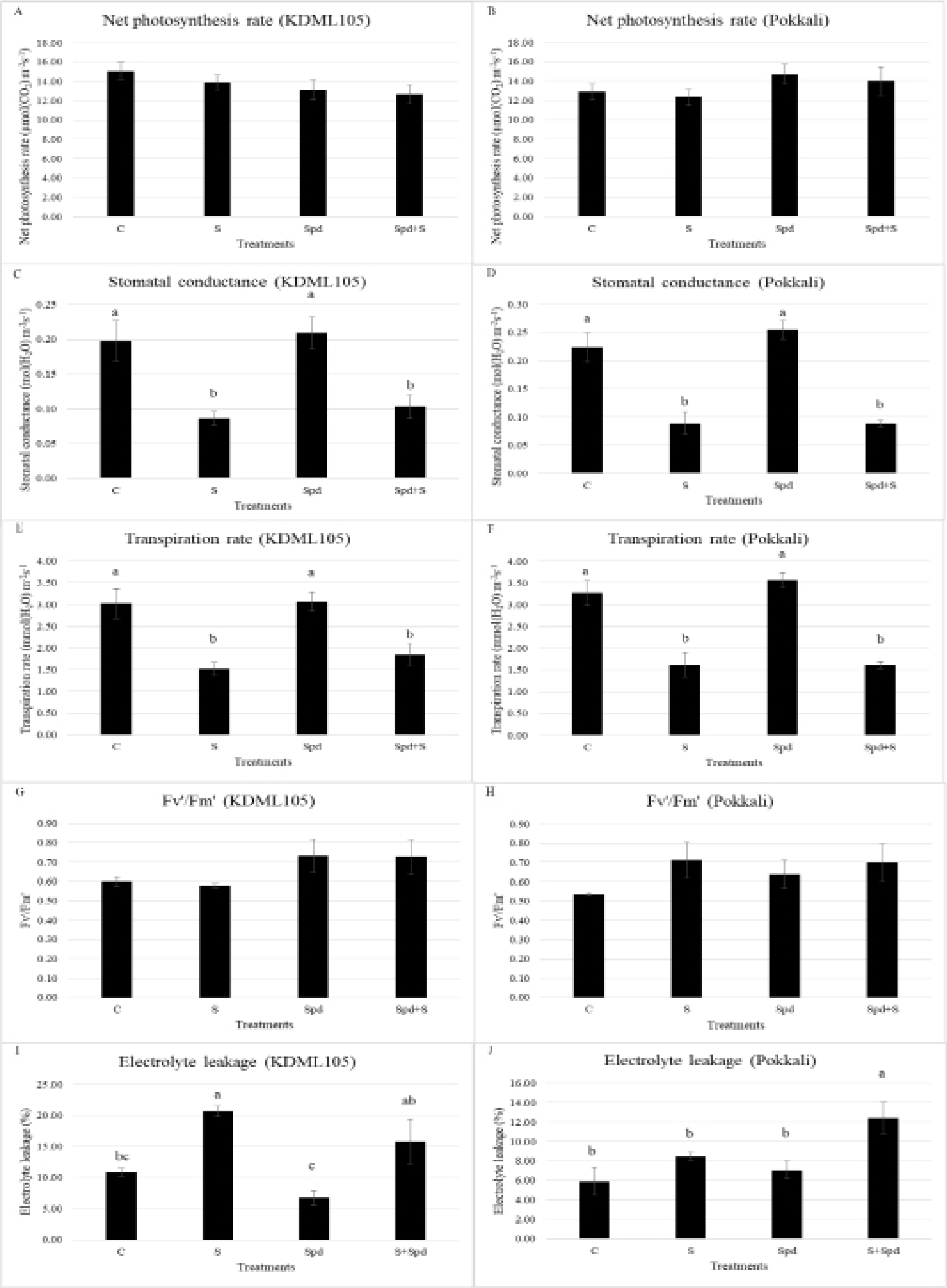

Figure 2 Effects of salinity on net photosynthesis rate (A: KDML105, B: Pokkali), stomatal conductance (C: KDML105, D: Pokkali), transpiration rate (E: KDML105, F: Pokkali), Fv´/Fm´ (G: KDML105, H: Pokkali) and electrolyte leakage (I: KDML105, J: Pokkali) and alleviation of salt-stress effects by exogenous Spd.C, control non-stress; S, salt-stress without Spd pretreatment; non-stress with Spd pretreatment; S+Spd, salt-stress with Spd pretreatment. [mean ± SE, means with different letters were significantly different at $p<0.05$]

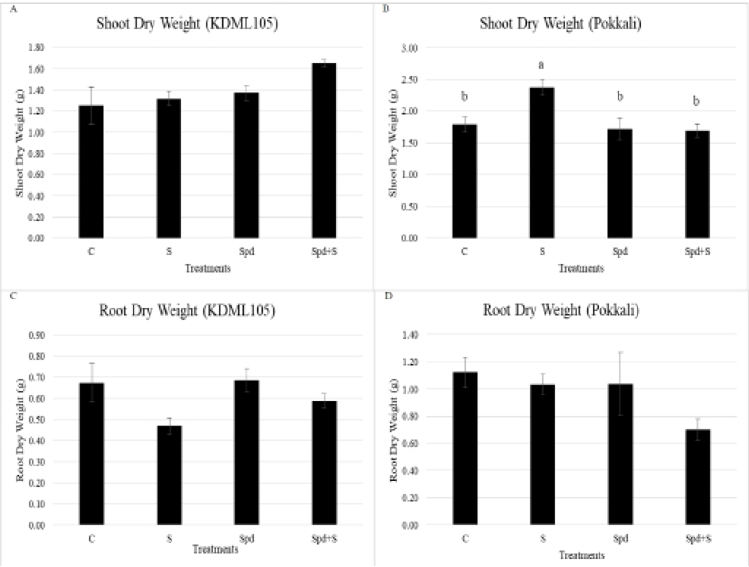

Figure 3 Effects of salinity on shoot dry weight (A: KDML105, B: Pokkali) and root dry weight (C: KDML105, D: Pokkali) and alleviation of salt-stress effects by exogenous Spd. C, control non-stress; S, salt-stress without Spd pretreatment; Spd, non-stress with Spd pretreatment; Spd+S, salt-stress with Spd pretreatment.[mean ± SE, means with different letters were significantly different at $p<0.05$]

Discussion and Conclusion

Generally, salt stress reduces photosynthesis of crops (Chaves et al., 2009). However, in this study, net photosynthesis rates of the plants at the vegetative stage were only slightly reduced from control plants and the efficiency of PSII photochemistry did not seem to affected. It has been reported that the level of salt sensitivity in rice varied with different growth stage being most sensitive at seed germination and young seedling stage, becoming more tolerant at the vegetative stage, and sensitive again at the reproductive stage (Heenan et al., 1988). Salt sensitivity also varied with salinity levels and duration of salt exposure. At the moderate level of salinity imposed in this study, the plants could be able to effectively partition toxic sodium ions to leaf sheath and old leaves, and to exclude them from young actively growing leaves (Munns and Tester, 2008). Therefore, the level of sodium ions in the green metabolically active leaves did not reach the level high enough to adversely inhibited chloroplast functions. Although salinity effects on photosynthesis process were not obvious, transpiration rates were significantly reduced as a result of lowered stomatal conductance. It has been reported that salinity stress reduced stomatal conductance in rice (Dionisio-Sese and Tobita, 2000) which in turn lowered transpiration rate to reduce water loss (Nounjan et al., 2016). Salinity obviously caused membrane damage, as a result of salt-stress induced ROS accumulation which destroyed membrane lipids (Gill and Tuteja, 2010), resulting in significant increase in electrolyte leakage particularly in KDML105. It has been reported that rice varieties with higher level of salt tolerance had greater antioxidant capacity, hence less ROS-induced membrane damage (Demiral and Türkan, 2005). It can be seen from **Figures 1 and 3** that application of exogenous spermidine could alleviate salt-stress effects and enhance growth of KDML105. Similar results were reported by Saleethong et al. (2011) and Chunthaburee et al. (2014) that exogenous Spd alleviated salt stress in rice by reducing ROS and enhancing activities of ROS-scavenging enzymes. Protective role of spermidine was also previously reported to be more pronounced in sensitive rice cultivar than in more tolerant ones (Roychoudhury et al., 2011). In conclusion, salt stress inhibited growth of sensitive rice KDML105 more than the tolerant variety Pokkali, and the effects of Spd on alleviation of salt stress damage was also more effective in KDML105 than Pokkali.

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