# The Effect of Exogenous Spermidine and Wood Vinegar on Growth and Physiology of Rice (*Oryza sativa* L.) cv. RD6 under Salt Stress

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## Abstract

Rice cv. RD6 is one of the most important crops for Thailand particularly for people and farmers in the northeastern part of the country. Saline soil in the northeastern part of Thailand is an important problem that causes low yield of rice. Treatment of plants with some plant growth regulators (PGR) such as polyamine or bio-stimulants, for example wood vinegar (WV), can induce physiological response so that plants become more tolerant to abiotic stresses including salinity. The objective of this study was to investigate the effects of spermidine (Spd) and WV on increasing tolerance of rice seedlings in saline soil under greenhouse condition. Rice seedlings were grown for 30 days in pots containing 5 kg soil. The plants were then sprayed with distilled water (control), WV (1:500), Spd (0.05, 0.1 and 0.5 mM) and mixture between WV (1:500) and Spd (0.05, 0.1 and 0.5 mM) for 5 days and then exposed to salt stress (150 mM NaCl) for 14 days. The result indicated that salt stress decreased net photosynthesis rate, maximal quantum yield of PSII photochemistry ($F_v/F_m$), but increased membrane damage as indicated by increase in electrolyte leakage (EL). Under salt stress, spraying with WV (1:500) and 0.1 mM Spd (with or without WV) tended to increase shoot and root growth, respectively. Spraying with 0.05 mM Spd and 0.5 mM Spd significantly reduced EL and increased $F_v/F_m$, respectively. The most effective solutions to promote shoot growth, root growth, reduce membrane damage and improve photochemical function of PSII were WV (1:500), 0.1 mM Spd, 0.05 mM Spd and 0.5 mM Spd, respectively.

**Keywords:** spermidine, wood vinegar, salt stress, photosynthesis, photosystem II, electrolyte leakage, glutinous rice
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## 1. Introduction

Rice is one of the main crops of Thailand and especialy in the northeastern part of the country. Saline soil is an important problem in the northeastern part of Thailand that causes low yield of rice [1]. Rice cv. RD6 is moderately sensitive to salinity [2]. Saline soil is also one of the most important factors in reducing yields of other crop species in many countries of the world.

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The problem of salinity is intensified because agricultural areas affected by salinity tend to increase. Global warming, deforestation, salt making and irrigation systems will greatly increase areas of salt-affected soils. For all over the world, 45 million hectares of irrigated land have been damaged by salt [3]. There are around 19.7 million rais or 3.2 million hectares of saline soils in Thailand, of which 17.8 million rais are found in the northeastern region. Spreading of salinity in northeast of Thailand has been caused by both nature and human activities. The natural causes of soil salinity included decay of salt rock at 1-2 m deep and evaporation of groundwater which brings salt to the surface. Human causes in the last 50 years included salt mining, deforestation and building irrigation systems and water resources on saline soils [1].

Salinity induced morphological and physiological changes including a reduction in the dry weight of leaves and roots, leaf area, root length, root volume, average root diameter, total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, net photosynthesis and stomatal conductance and increasing in proline and MDA content [4-6]. In addition, salinity caused ion imbalance in plants as shown by higher $Na^+$ and $Cl^-$ contents and lower $K^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations [4, 6-8]. These imbalances of the cellular ions result in ion toxicity, osmotic stress and production of reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide and hydroxyl radicals. Salt-tolerant plants can regulate ion and water movements more efficiently than salt-sensitive plants and have a better antioxidant system for effective removal of ROS [9, 10]. These antioxidant systems include antioxidant enzymes for example superoxide dismutase, catalase, glutathione peroxidase and peroxidases and enzymes of ascorbate-glutathione cycle including ascorbate peroxidase and glutathione reductase. These antioxidant enzymes will help to decrease salt stress effect from oxidation processes of biomolecules [11].

Polyamine is one of plant growth regulators consisting of putrescine (Put), spermidine (Spd) and spermine (Spm) which can alleviate the effects of abiotic stresses on plants. Exogenous Spd has been reported to improve plant tolerance to abiotic stresses including salinity [12]. Spraying with Spd on rice leaves could also alleviate oxidative stress on rice [13]. Moreover, diluted wood vinegar (WV) could promote growth under salt stress of rice seedlings [14]. The objective of this study was to examine the effect of two plant growth regulators including Spd and WV on alleviation of salt-stress damages in rice under salt stress.

## 2. Materials and Methods

### 2.1 Plant materials and treatment

Seeds of rice cv. RD6 were obtained from Khon Kaen Rice Research Center, Department of Rice, Thailand. The experiment was performed in a greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University. Seeds were surface-sterilized with 15% sodium hyperchlorite for 15 min, then rinsed 5 times with distilled water. The seeds were germinated in dark conditions for 3 days. Seedlings were then planted in pots containing 5 kg of dry soil from paddy field. The soil was loamy sand comprising 0.55% organic matter, 0.045% Nitrogen, 0.32 mg $kg^{-1}$ Phosphorous, 25.21 mg $kg^{-1}$ Potassium, 68.70 mg $kg^{-1}$ Sodium and 11.50 mg $kg^{-1}$ Chlorine, having a slightly alkaline pH at 6.55 and electrical conductivity at 0.045 dS $m^{-1}$. When rice seedlings were 30 days old, they were subjected to 8 spraying treatments as follows: distilled water (control), diluted wood vinegar (WV) (1:500), Spd solutions (0.05, 0.1, 0.5 mM) and mixture between WV (1:500) and Spd solutions (0.05, 0.1, 0.5 mM) using 50 ml of solutions per pot during 17.00-18.00 for 5 days. The dilution of WV and concentrations of Spd used were based on previous studies [14, 15]. Afterwards, salt stress was imposed by watering with 100 mM NaCl solution (salt-stressed group) for 3 days. Then, NaCl concentration was increased to 150 mM. Another set of plants also received

8 spraying treatments but continued to be fed with regular watering (non-stressed group). Experimental design was completely randomized with five pots for each spraying treatment and each pot contained 12 plants.

### 2.2 Measurement of plant growth

Growth data and leaf physiological parameters were collected at 14 days after adding sodium chloride. Plant growth parameters measured included shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot length and root length. For dry weight measurement, all samples were dried in hot air oven at 70 °C for 72 h.

### 2.3 Measurement of physiology

Physiological measurements included net photosynthetic rate ($P_N$) by using an infra-red gas analyzer (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 and $CO_2$ concentration at 400 µl $l^{-1}$, chlorophyll fluorescence parameters by chlorophyll fluorometer (HandyPEA, Hansatech Instruments), relative water content (RWC) by using the following formula RWC=(FW-DW)/(TW-DW) × 100, where FW, DW and TW was initial fresh weight, dry weight and fully turgid weight, respectively. Membrane stability was determined by measuring electrolyte leakage percentage (EL) by cutting fresh leaves sample into 1 $cm^2$, placing 6 pieces in closed vials containing 10 ml of deionized distilled water, incubating at 25 °C for 24 h in the dark and measure electrical conductivity (EL1). Then, the vials containing leaf pieces were boiled (100 °C) for 15 min and left to cool down to room temperature, electrical conductivity of the solution was measured to obtain EL2. EL (%) was estimated as: (EL1/EL2) × 100.

### 2.4 Statistical analysis

SPSS ver. 23 was used for statistical analysis. Data were presented as means ± SE. Significant difference among the treatment means was determined by Duncan's multiple range test (DMRT) ($p \leq 0.05$). Each treatment had five replications and this experiment was designed by using completely randomized design (CRD).

## 3. Results and Discussion

As shown in Table 1, plants exposed to salt stress for 14 days did not show significant reduction in growth parameters compared with those of the non-stress plants. Among the non-stressed groups, only plants sprayed with 0.5 mM Spd+WV had significantly higher growth (shoot and root fresh weight, FW and shoot dry weight, DW) than the control plants sprayed with $H_2O$. The solution 0.5 mM Spd+WV induced the highest shoot FW (7.63 g $plant^{-1}$) and root FW (8.28 g $plant^{-1}$) which were 45.33 and 53.90% increase from the plants sprayed with $H_2O$. In addition, plants sprayed with diluted WV (1:500) had significantly higher shoot DW (1.64 g $plant^{-1}$) than that of the control (1.17 g $plant^{-1}$). For the plants exposed to NaCl stress, most spraying treatments tended to increase growth (fresh and dry weights) compared with plants sprayed with $H_2O$ but the differences were not statistically significant. It is noted that, among salt-stress groups, spraying with diluted WV resulted in highest shoot FW (7.32 g $plant^{-1}$) and shoot DW (1.73 g $plant^{-1}$) (although not significantly different from the plants sprayed with water). WV was the most effective solution that induced the highest shoot growth (both FW and DW) for NaCl-treated plants. For root growth, highest FW was recorded for 0.1 mM Spd, and highest DW for 0.05 mM Spd. Similar with Shunkao and Theerakulpisut [16] suggested that foliar spraying with Spd could alleviate salt-stress effects and enhance growth of rice cv. KDML105. Chunthaburee *et al*. [15] also reported that Spd priming improved growth of rice seedling when plants were exposed to salt stress. Moreover, Theerakulpisut *et al*. [14] demonstrated that seed priming with wood vinegar could also alleviate the effect of salt stress on growth in rice cv. KDML105.

Salinity stress damaged plant membranes as indicated by dramatic increase in electrolyte leakage from 6.3-16.3% in the non-stressed group to 48.1-75.7 % in the salt-stressed group (Figure 1A). Under non-stressed condition, spraying with Spd and WV demonstrated no significant difference as compared with water spraying. Under salt stress condition, spraying with 0.05 mM Spd was able to significantly decrease EL from 75.7 % (sprayed with $H_2O$) to 48.1%. Similar findings by Shunkao and Theerakulpisut [16] showed that foliar spray with Spd also reduced membrane damage in leaves of salt-stressed rice cv. KDML105.

Salinity caused osmotic effects and salt-stressed plants had lower RWC than non-stressed plants (Figure 1B). Plants treated with salts had lower RWC than non-stressed ones. However, for both non-stressed and salt-stressed conditions, RWC of plants sprayed with all Spd and WV treatments did not differ significantly from that of the controls (sprayed with water). Our results are in agreement with Bassiouny and Bekheta [17] who reported that salt stress decreased the RWC of wheat.

**Table 1.** Effect of foliar spraying with wood vinegar (WV), spermidine (Spd) and mixture of both, on growth of rice plants after exposure to sodium chloride (150 mM NaCl) for 14 days.

| **Treatment** | **Lengths (cm)** | | **Fresh weights (FW) (g plant$^{-1}$)** | | **Dry weights (DW) (g plant$^{-1}$)** | |
|---|---|---|---|---|---|---|
| | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot** | **Root** |
| **Non-stressed ($H_2O$)** | | | | | | |
| $H_2O$ | 69.6abc | 21.0a | 5.25bc | 5.38bcd | 1.17cd | 0.80abc |
| WV (1:500) | 71.6a | 24.9a | 7.42ab | 8.08ab | 1.64ab | 1.11a |
| 0.05 mM Spd | 66.1abc | 24.2a | 5.99abc | 7.14abcd | 1.31abcd | 0.96ab |
| 0.1 mM Spd | 70.7ab | 26.5a | 6.92abc | 7.60abc | 1.48abcd | 0.96ab |
| 0.5 mM Spd | 68.3abc | 26.3a | 5.59abc | 6.20abcd | 1.24bcd | 0.86abc |
| 0.05 mM Spd+WV | 68.4abc | 22.1a | 4.85c | 5.51bcd | 1.08c | 0.72bc |
| 0.1 mM Spd+WV | 71.6a | 26.0a | 6.96abc | 7.82abc | 1.60abc | 1.09a |
| 0.5 mM Spd+WV | 71.4a | 24.7a | 7.63a | 8.28a | 1.69ab | 1.09a |
| **Salt-stressed (NaCl)** | | | | | | |
| $H_2O$ | 67.6abc | 21.6a | 5.60abc | 4.55c | 1.28abcd | 0.52c |
| WV (1:500) | 66.7abc | 21.3a | 7.32ab | 5.52bcd | 1.73a | 0.65bc |
| 0.05 mM Spd | 64.8bc | 24.6a | 6.47abc | 6.36abcd | 1.43abcd | 0.71bc |
| 0.1 mM Spd | 64.2c | 26.0a | 6.51abc | 6.58abcd | 1.36abcd | 0.65bc |
| 0.5 mM Spd | 65.9abc | 25.9a | 5.45abc | 5.92abcd | 1.29abcd | 0.58c |
| 0.05 mM Spd+WV | 64.9bc | 24.8a | 6.20abc | 6.12abcd | 1.39abcd | 0.63bc |
| 0.1 mM Spd+WV | 66.3abc | 25.7a | 6.08abc | 5.96abcd | 1.31abcd | 0.84abc |
| 0.5 mM Spd+WV | 66.5abc | 22.5a | 7.16ab | 5.29bc | 1.58abc | 0.68bc |

*Means in the same column sharing same alphabets were not significantly different at $P \leq 0.05$

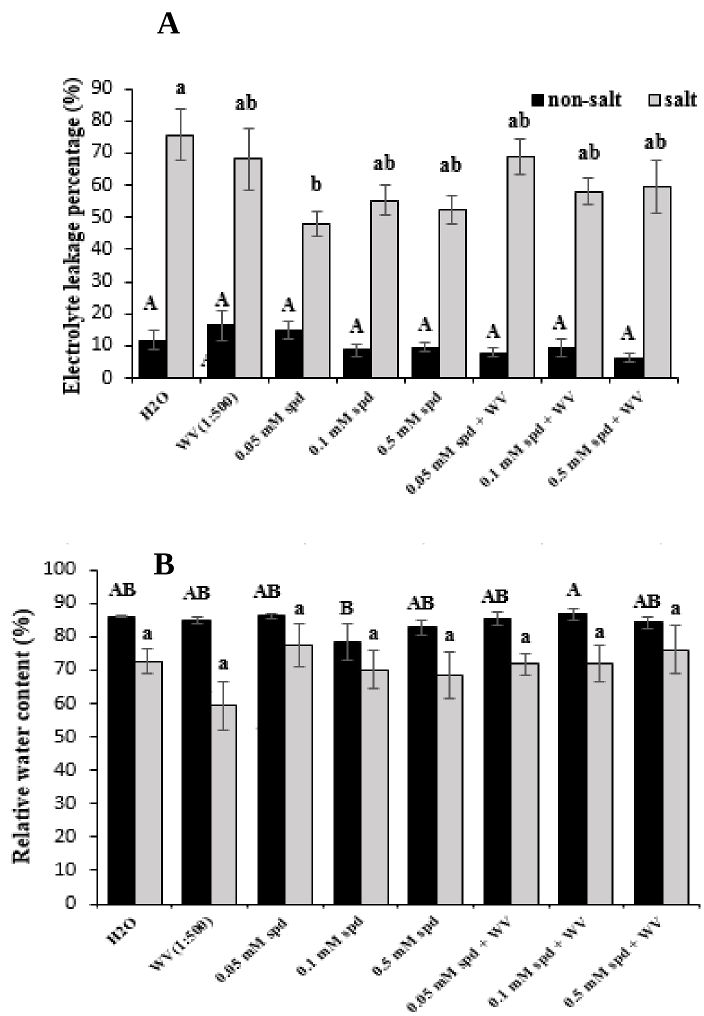


**Figure 1.** Effect of spraying with spermidine and wood vinegar on electrolyte leakage percentage (A) and relative water content (B) after giving sodium chloride (150 mM NaCl) for 14 days. [mean±SE, means sharing same alphabets (uppercase, non-saline condition; lowercase, saline condition) were not significantly different at $P \leq 0.05$].

Salt stress decreases photosynthesis of crops by inducing stomatal closure and hence reducing $CO_2$ diffusion or by alteration of photosynthetic metabolism as a result of ion toxicity [18]. Salt treatment could reduce photosynthesis in *Populus euphratica* [19], *Citrus reshni* [20] and *Oryza sativa* [21]. In this study, net photosynthesis rates under non-stressed conditions ranged from 22.1 to 25.1 μmol $CO_2$ $m^{-2}$ $s^{-1}$ while those of the stressed plants reduced from 7.5 to 11.6 μmol $CO_2$ $m^{-2}$ $s^{-1}$. Spraying with 0.1 mM Spd+WV, 0.5 mM Spd+WV and 0.5 mM Spd could alleviate effects of salt stress on photosynthesis by causing 36.5, 28.2 and 28.2%, respectively increase (although non-significantly different) in net photosynthesis rates compared with water spraying. In non-stressed condition, spraying with 0.05 mM Spd+WV, 0.1 mM Spd+WV and 0.5 mM Spd+WV can cause non-significant increase in net photosynthesis rates (9.6%, 5.2% and 4.8%, respectively) compared with water spraying (Figure 2A). Similar to Anjum [22], adding 0.5 mM Spd to the NaCl nutrient solution could significantly increase net photosynthesis rate of Troyer citrange stressed with 75 mM NaCl. In addition, foliar spraying with 0.5 mM Spd could increase net photosynthesis rate of rice cv. Pokkali under salt stress [16].

Salt stress affected function of chloroplast photosystems by reducing maximal quantum yield of PSII photochemistry (Fv/Fm) [23]. Similar to this study, salt stress reduced Fv/Fm of plants sprayed with $H_2O$ (from 0.827 to 0.812) and spraying with 0.5 mM Spd significantly increase Fv/Fm of salt-stressed plants to 0.821 (Figure 2B). The remaining spraying treatments also increased Fv/Fm without any significantly difference from water spraying.

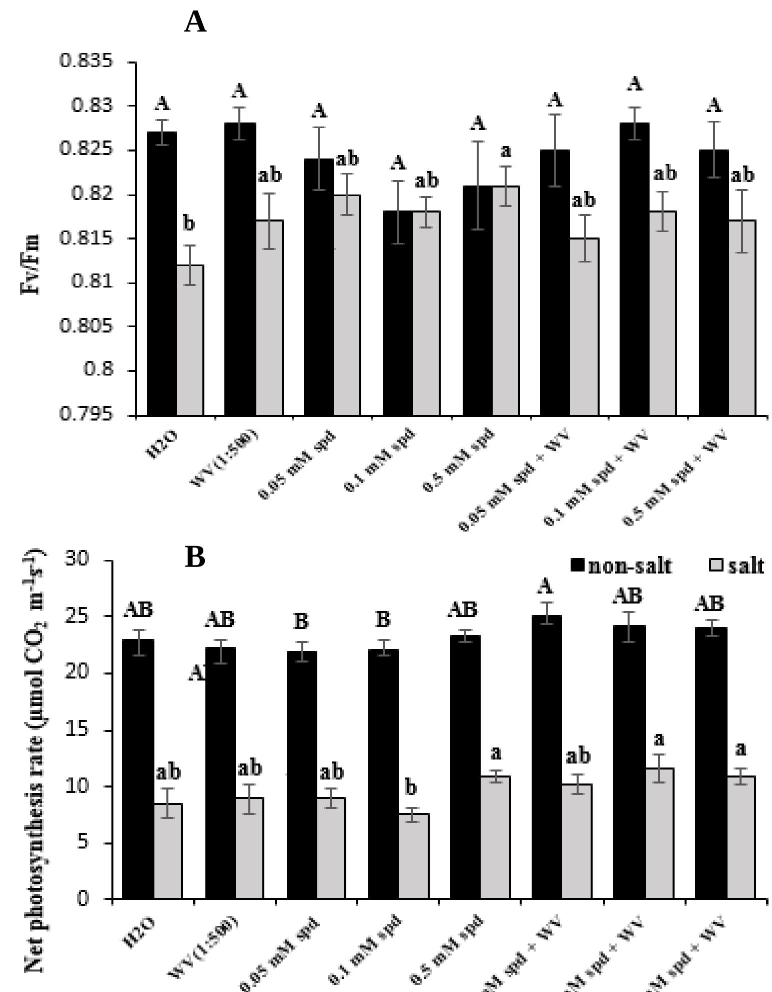


**Figure 2.** Effect of spraying with spermidine and wood vinegar on net photosynthesis rate (A) and Fv/Fm (B) after giving sodium chloride (150 mM NaCl) for 14 days. [mean±SE, means sharing same alphabets (uppercase, non-saline condition; lowercase, saline condition) were not significantly different at P≤0.05].

## 4. Conclusions

Under salt stress, foliar spraying with diluted WV tended to promote shoot growth of rice cv. RD6 while spraying with 0.1 mM Spd (with or without WV) had a tendency to increase root growth. The physiological characteristics showed that salt stress increased EL, and reduced RWC, $P_N$ and $F_v/F_m$. Foliar spraying with 0.05 mM Spd and 0.5 mM Spd alleviated adverse effects of salt stress by reducing EL and increasing Fv/Fm, respectively. For practical application, the effectiveness of Spd and WV on alleviation of salt stress in rice should be further explored in the field condition.

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