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EXOGENOUS APPLICATION OF SPERMIDINE (SPD) AND WOOD VINEGAR IMPROVES SALT TOLERANCE IN SALT-SENSITIVE RICE (*ORYZA SATIVA* L.)

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Abstract

Spermidine (Spd) and wood vinegar are widely reported for its positive role to ameliorate plants under abiotic stress. In this study, the protective roles of Spd and wood vinegar in salt-sensitive rice cultivars 'Khao Dawk Mali 105 (KDML105)' and 'RD6' exposed to salinity stress were investigated under greenhouse and field conditions by dividing into two experiments. Under the greenhouse condition, three concentrations of Spd (0.1, 0.5 and 1.0 mM) were sprayed daily to 60-d-old 'KDML105' plants (booting stage) grown in soil for 7 d ahead of watering the plants with tap water (control) or 30 mM NaCl (salt stress). The results showed that spraying 0.5 mM Spd was most effective in improving the salt tolerance of 'KDML105' as evidenced by an increase in the development of flag leaves, SPAD values, yield and yield components. To reduce crop production cost, the second experiment was conducted. The concentration of Spd used was based on the greenhouse experiment. Exogenous Spd (0.5 mM) was applied in comparison with wood vinegar (1:300 and 1:500) under field condition. Rice 'KDML105' and 'RD6' were grown under low (EC = 3 dS m^{-1}) and high (EC = 10 dS m^{-1}) saline fields. At the booting stage, Spd or wood vinegar were sprayed daily for 7 d. In both saline field sites, both rice cultivars sprayed with Spd and/or wood vinegar at all tested ratios showed a marked increase in yield and yield components, with better results observed for 'KDML105'. The findings suggest that Spd and wood vinegar could be applied for alleviating the effects of salt stress on rice production.

Key words: Rice, Salt stress, Spermidine, Saline soil, Wood vinegar.

Introduction

Soil salinity is one of the limiting factors for agriculture causing yield reduction in major crops by over 50% (Abdallah *et al*., 2016). It has been reported that more than 6% of land throughout the world are salt-affected (Munns, 2005). Thailand is one of the countries which has saline soil problems, especially in the northeastern region. Approximately 17% of the total land in this area is affected by soil salinity (Yuvaniyama, 2004). Direct effects of salts in soils include induction of osmotic stress due to low soil water potential, and ion toxicity from Na and Cl ions. High salt concentrations in plant tissues also lead to accumulation of reactive oxygen species (ROS) resulting in oxidative stress which consequently causes membrane damage, reduction in photosynthesis, slower growth and poor yield (Ghosh *et al*., 2011; Saleethong *et al*., 2016).

As a glycophyte by nature, rice is highly susceptible to soil salinity in most developmental stages throughout its life, from seed germination, vegetative growth, to reproductive development (Saleethong *et al*., 2016). While seedlings of rice have been reported to die at a salt level of 10 dS m^{-1} (Munns *et al*., 2006), rice yield is reduced by 50% at a salt level of 6.9 dS m^{-1} (Grattan *et al*., 2002).

Salinity causes significant yield loss in rice crops particularly when salinity is imposed at the flowering stage. Rice genotypes with higher level of salt tolerance exhibited high K^+ and low Na^+, and grain weight had significant positive correlation with K^+/Na^+ ratio (Shereen *et al*., 2020). Salt tolerance in rice involves a variety of adaptive mechanisms, including retrieving Na^+ from root xylem to reduce transport of Na^+ to shoots, stimulating efflux of Na^+ from root cells back to soil and from shoot tissues into translocation stream, compartmentalization of Na^+ into the vacuoles, increasing accumulation of compatible solutes and ROS scavenging molecules (Reddy *et al.*, 2017). Unfortunately, due to the fact that the threshold for most cultivated crops including rice, a moderately salt-sensitive cereal, to survive is at the salt level of about 3 dS m^{-1} (Hoang *et al.*, 2015). This is one of the reasons why millions of hectares of salt-affected land in South and Southeast Asia, where climate is most suitable for rice growing, are left uncultivated or used to grow crops with very low yields (Reddy *et al.*, 2017). Thus, development of strategies for increasing salt tolerance or ameliorating salt-induced damages in rice is a great challenge.

In the past few decades, many studies have been devoted to the development of salt-tolerant rice cultivars either by molecular breeding or transgenic technology (Bimpong *et al.*, 2016; Reddy *et al.*, 2017). However, none of the proposed technologies has been found to show full effectiveness under saline soil conditions due to complex nature of salt tolerance mechanisms, lack of efficient selection criteria, and interference of other environmental factors rather than salt stress (Flowers, 2004). Accordingly, other alternative approaches to reduce negative impact of salt stress in rice should be developed.

Exogenous application of polyamines (PAs) like spermidine (Spd) has been widely reported for its protective roles to alleviate adverse effects of salt stress in plants (Roychoudhury *et al.*, 2011; Chunthaburee *et al.*, 2015a). Exogenous Spd has been reported to prevent electrolyte leakage, recover plasma membrane damage in rice under salt and chilling stresses (Roychoudhury *et al*., 2011; Zeng *et al*., 2016). Spd also acts as a scavenger of free radicals induced by salt stress (Du *et al*., 2010). Based on these observations, the protective role of exogenous Spd in rice grown under saline soil conditions is of particular interest. In spite of a plethora of studies of positive effects of Spd application in a variety of rice genotypes under saline conditions, most of the studies focused on the protective role of Spd at a particular stage of growth and development under greenhouse conditions, not covering all growth and development stages or not examining crop yield under field conditions (Chunthaburee *et al*., 2015a; Saha & Giri, 2017). Investigation of the effects of exogenous Spd on growth and development, and yield of rice genotypes under field conditions is of great importance.

Pyroligneous acid or wood vinegar is an acidic reddish-brown aqueous liquid which is a byproduct of the carbonization process of woods or wood residues. It has been utilized in agriculture to promote plant growth. Over 200 water-soluble compounds including organic acids, phenolics, alkanes, alcohols and esters are detected in wood vinegar (Wu *et al*., 2015). Wood vinegar has been reported to promote field emergence and increase drought tolerance ability in rice when used as seed priming agents (Dissatian *et al*., 2018). Taking into account the beneficial effects on plant growth, wood vinegar can possibly be used as plant growth regulator to enhance growth and yield of rice plants under salt stress conditions.

For this purpose, this study was undertaken to investigate the effects of exogenous Spd and wood vinegar on growth and yield of two salt-sensitive rice genotypes, ‘KDML105’ and ‘RD6’, under greenhouse and field conditions.

Materials and Methods

Plant materials: Seeds of the salt-sensitive rice ‘KDML105’ and ‘RD6’ were kindly provided by Khon Kaen Rice Research Center, Bureau of Rice Research and Development, Khon Kaen province, Thailand.

Greenhouse experiments: Greenhouse experiments were carried out at the Field Crop Research Station, Faculty of Agriculture, Khon Kaen University (Thailand), to study the effects of exogenous Spd on agronomic traits and yield of ‘KDML105’. The type of soil used in the experiment was sandy loam. The chemical and physical properties of the soil are presented in Table 1. The soil was sieved through a 2-mm mesh and 2 kg of the soil was weighed and put into a plastic pot (20-cm diameter).

Rice seedlings were planted in plastic pots, one seedling per pot, and irrigated with tap water, with the water level kept 5 cm above the soil surface. After 60 days of planting or at the booting stage, Spd mixed with Tween-20 as a wetting agent was applied exogenously to rice leaves at 0, 0.1, 0.5 and 1.0 mM on a daily basis for 7 days (05.00-06.00 pm) prior to salt-stress treatments, in which rice plants were irrigated with 30 mM NaCl (salt stress) or tap water (control) until harvest. Fertilizers were applied twice, while weeds were removed by hand and pesticides were used only when required. A completely randomized design (CRD) was used, with 5 replicates for each treatment, and one plant per replicate. Sampling was performed at 10-day intervals to determine changes in the physiological characteristics.

Leaf greenness value or SPAD reading was measured on the leaf tip, mid leaf and leaf base regions of flag leaves, second leaves, and third leaves, using a chlorophyll meter (SPAD-502, Minolta Camera Co., Japan). After 30 days of salt stress treatment, flag leaf length, width and area were assessed using a model CI-203 Handheld Laser Leaf Area Meter (CID Bio-Science Inc., USA). At harvest or 60 days after salt stress treatment, yield and yield components, which included plant height, number of tillers per pot, panicle length, % seed setting, % unfilled grain, 100-grain weight, number of filled grains per pot, filled grain weight per pot, root dry weight, straw dry weight, panicle weight, above ground weight, biomass and harvest index, were measured. Moreover, 10 grains were randomly sampled from each treatment to measure the width and length of hulled grains and kernels using a vernier caliper.

Table 1. Chemical and physical properties of the soil used for the greenhouse experiment.

Parameters	Contents	Methods
Chemical properties		
pH	6.43	1:1
EC (dS/cm)	0.04	1:5
LR (Kg $CaCo_3$/rai)	188.00	Woodruff method
OM (%)	0.49	Walkey and Black method
Total N (%)	0.03	Kjeldahl method
Na (ppm)	37.15	Atomic absorption spectrophotometry
Cl (ppm)	27.00	Water extraction and titrated with silver nitrate
P (ppm)	69.64	Bray no. 2 extraction and molybdenum blue
K (ppm)	171.27	Atomic absorption spectrophotometry
CEC (c mol/kg)	3.00	Ammonium saturation
Physical properties		
Field capacity (%)	23.51	
Sand (%)	60.73	
Silt (%)	30.11	
Clay (%)	9.16	
Soil texture	sandy loam	

Field experiments: The experiments were conducted at a farmer's field located in Ban Nong Koi, Dang Yai sub-district, Muaeng district, Khon Kaen, Northeast Thailand (16º28'19.4"N – 102º42'25.2"E and 178 m altitude). This area is considered as rain-fed lowland with total seasonal rainfall mainly during May to September of around 943.0 mm. The soil of the field site is sandy loam. The field site can be categorized into two major zones: a low saline soil zone with an electrical conductivity (EC) of 3 dS m^{-1} and an alkaline pH of 8.0, and a high saline soil zone with an EC of 10 dS m^{-1} and an alkaline pH of 8.2. This high saline site has been left uncultivated for years due to the severity of soil salinity. Rice plants (aged 30-45 days) of two salt-sensitive rice cultivars 'KDML105' and 'RD6' were used to test the objectives. Because rice plants exhibiting comparable tolerance to salinity at certain EC values have been documented to display striking variations in salt tolerance at elevating EC values (Suriya-arunroj *et al*., 2005), differences in the alleviating effects of exogenous Spd and wood vinegar on yield and yield components can possibly be detected under field conditions at different EC values, and therefore, the salt-sensitive 'RD6' was included for comparison.

At the booting stage, rice plants of each cultivar were foliar-sprayed with 0.5 mM Spd or wood vinegar (1:300 and 1:500 dilutions selected based on our previous study (Theerakulpisut *et al*., 2016)) on a daily basis for 7 d during 05.00-06.00 pm. Fertilizers were applied twice throughout the whole study period. Wood vinegar was included for comparison because it was reported that it could enhance growth of rice and tomato (Kulkarni *et al*., 2006; Mungkunkamchao *et al*., 2013).

The experimental design was randomized complete block design (RCBD) with four replicates. Each treatment had twenty plants per replicate. Each treatment consisted of two rows, each containing ten rice plants planted with a spacing of 20 × 20 cm between plants. The distance between treatments was 0.5 m and the field ridge was built 60 cm above the ground to prevent the contamination of the foliar-applied substances. At harvest, five rice plants were randomly taken from each treatment to determine crop yield and yield components.

Statistical analysis

All data were analyzed using the statistical package SPSS 17.0 to test the significance of treatments. The results are presented as the mean ± SE. One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to determine the significance relative to the control. In all cases, significant difference was considered at $p<0.05$.

Results and Discussion

Effects of exogenous Spd on physiological characteristics, yield and agronomic traits of rice 'KDML105' plants grown on saline soil under greenhouse conditions: Flag leaves of rice plants have been reported to be a major source providing photoassimilates and minerals for development of spikelets and seeds of rice (Zhang *et al*., 2010). Thus, it is important to evaluate their growth and development under salt stress conditions. In this study, salinity was found to impose a small adverse effect on the growth of flag leaves as observed for rice plants grown under greenhouse conditions, causing a slight reduction in leaf area and width. This slight reduction in the growth of flag leaves might be due to the low severity of salinity (30 mM NaCl), which was much lower than that observed in other studies (Thitisaksakul *et al*., 2015; Wankhade *et al*., 2013). However, the specific leaf area of flag leaves which directly reflects the leaf thickness was remarkably reduced (17%) under this low-severity salinity (Table 2), implying a decrease in cell size, chlorophyll and protein contents per leaf area. Our results suggested that specific leaf area should be included for selection of rice for salt tolerance under greenhouse conditions since this character was found to be considerably affected by salinity even at low levels. As expected, exogenously applied Spd ameliorated the negative effects of salinity stress through improvements in the growth of flag leaves. When compared to the foliar application of Spd at 1.0 mM under salt stress conditions, spraying rice plants with 0.5 mM Spd was found to be more cost-effective in alleviating salinity stress, in which the leaf area, leaf length, leaf width, leaf length/leaf width ratio, and specific leaf area of flag leaves were increased by 59, 47, 11, 35 and 8%, respectively (Table 2). The results obtained in our study were in good agreement with the study presented by Roychoudhury *et al*., 2011 which elucidated that 1.0 mM Spd alleviated the 200 mM NaCl-caused inhibition of root and shoot lengths of rice cultivars. Many other studies have also elucidated the beneficial role of exogenous Spd in improvement of salinity tolerance in rice as evidenced by the regulation of specific physiological processes, resulting in increased growth and yield (Saleethong *et al*., 2013; Chunthaburee *et al*., 2015a, b; Liu *et al*., 2015). Furthermore, under salinity stress wheat plants raised from grains pretreated with 0.3 mM spermine (Spm), one of the most common PAs, were found to display an increase in the flag leaf area at ear emergence as compared to the control plants (Aldesuquy *et al*., 2014).

In this study, flag leaf greenness which reflects the leaf chlorophyll contents was measured and expressed as the SPAD values. Our results revealed that flag leaves taken from salt stressed plants exhibited an increase in the SPAD values during the first 20 days of salinity treatment, followed by a 15% decrease in the SPAD values detected on day 30 and leaf death was observed on day 50. Reduction in chlorophyll synthesis and induction of its degradation led to decreased flag leaf greenness. It has been reported that reduction in leaf greenness is caused from oxidative stress in salt-stressed plants (Djanaguiraman *et al*., 2006; Kaur *et al*., 2016; Taïbi *et al*., 2016).

After 40 days of salt stress SPAD values of flag leaves significantly reduced (Table 3). However, pretreatments of plants by spraying with all 3 concentrations of Spd significantly increased flag leaf greenness. The findings obtained in our study were in agreement with an earlier study which elucidated that treatment of rice seedlings with 1.0 mM Spd during salinity stress reduced the loss of chlorophyll (Chattopadhayay *et al*., 2002). Moreover, in another study, exogenous Spd was also found to protect chlorophyll loss, maintain membrane integrity, and mitigate the negative effects of salinity (Puyang *et al*., 2016).

Table 2. Effects of foliar sprays of Spd (0, 0.1, 0.5 and 1 mM) on growth characteristics of flag leaves on the main tillers of 'KDML105' rice plants observed at 30 days after salt stress treatment (DAT).

Treatment	Leaf area (cm^2)	Leaf length (cm)	Leaf width (cm)	Leaf length/width ratio	Specific leaf area ($cm^2\ g^{-1}$)
Control	20.81c	22.12c	1.25c	17.89c	182.07a-c
NaCl	19.00c	23.02c	1.24c	18.43bc	151.06d
0.1 mM Spd	32.20a	31.33ab	1.40bc	22.42ab	197.74ab
0.1 mM Spd + NaCl	26.51bc	27.12bc	1.30bc	20.66a-c	172.91cd
0.5 mM Spd	35.99a	33.13a	1.47a	23.05a	199.80a
0.5 mM Spd + NaCl	33.08a	32.58ab	1.35a-c	24.06a	202.90a
1 mM Spd	35.58a	33.27a	1.44bc	23.09a	194.80a-c
1 mM Spd + NaCl	35.12a	34.32a	1.36a-c	25.13a	174.52bc

*Means in the same column followed by different letters are statistically different at $p<0.05$

Table 3. Effects of foliar sprays of Spd (0, 0.1, 0.5 and 1 mM) on the greenness (SPAD values) of flag leaves of 'KDML105' rice plants observed at 10-50 days after salt stress treatment (DAT).

Treatment	10 DAT	20 DAT	30 DAT	40 DAT	50 DAT
Control	45.11ab	36.96c	24.15d	15.84b	18.88a-c
NaCl	46.13a	40.81a	20.62e	14.09b	n.d.
0.1 mM Spd	44.53ab	37.23bc	26.27cd	17.67ab	18.50a-c
0.1 mM Spd + NaCl	45.62ab	41.34a	30.34ab	22.15a	24.00a
0.5 mM Spd	44.29ab	38.47a-c	28.28bc	17.65ab	16.41bc
0.5 mM Spd + NaCl	43.73b	39.78a-c	32.84a	18.83ab	19.37a-c
1 mM Spd	44.54ab	41.56a	33.31a	21.63a	22.26ab
1 mM Spd + NaCl	44.07b	40.60ab	31.31ab	21.59a	24.59a

*n.d. = Not detected; means in the same column followed by different letters are statistically different at $p<0.05$

Table 4. Effects of foliar sprays of Spd (0, 0.1, 0.5 and 1 mM) on height, number of tillers per pot, panicle length, percent seed setting, percent unfilled grain, 100-grain weight of 'KDML105' rice plants observed at the end of salt stress treatment.

Treatment	Height (cm)	No. of tillers/ pot	Panicle length (cm)	Seed setting (%)	Unfilled grain (%)	100-grain weight (g)
Control	126.25bc	8.63ab	21.47b	68.80bc	31.20ab	2.68a
NaCl	122.78c	9.38a	21.98b	68.40bc	31.59ab	2.38bc
0.1 mM Spd	126.24bc	8.00a-c	22.76ab	73.99ab	26.01bc	2.66a
0.1 mM Spd + NaCl	130.01ab	8.75a	21.13b	65.70c	34.29a	2.35c
0.5 mM Spd	134.19ab	8.38a-c	23.88a	77.56a	22.43c	2.62a
0.5 mM Spd + NaCl	130.10ab	8.25a-c	23.78a	78.08a	21.91c	2.45b
1 mM Spd	131.56ab	6.88c	23.75a	78.34a	21.66c	2.66a
1 mM Spd + NaCl	131.61ab	7.13bc	24.36a	76.13a	23.87c	2.45b

*Means in the same column followed by different letters are statistically different at $p<0.05$

In this study, in the pot experiments, salinity was found to cause a significant decrease in 100-grain weight, filled grain weight per pot, straw weight, panicle weight, harvest index, seed length, and seed length/width ratio (Tables 4-6), and this reduction was alleviated by exogenously applied Spd. Our findings were in agreement with an earlier study which demonstrated the improved growth and yield of 30 mM NaCl-stressed plants of rice triggered by 10 µM Spd (Ndayiragije & Lutts, 2007). Overall, based on pot experiments in our study, salinity was found to impose deleterious effects on rice growth and yield and yield components, and foliar application of 0.5 mM Spd was observed to be most cost-effective in improving the crop performance and was therefore chosen for field experiments.

Effects of exogenous Spd and wood vinegar on yield and yield components of rice 'KDML105' and 'RD6' grown under saline soil field conditions: Field experiments were performed under low and high saline soil conditions to compare the beneficial effects of exogenous Spd and wood vinegar in two rice cultivars ('KDML105' and 'RD6'). It was observed that 30-day-old rice plants planted on the first attempt failed to maintain viability in a high saline soil field with visible leaf yellowing/browning and subsequent death was detected only a week after transplanting, indicating the high-severity salinity of the field, and 45-day-old plants were thus planted instead (data not shown). The findings obtained in our study are consistent with the results of an earlier study which documented that soil salinity showed a considerable deleterious effect on the grain yield of rice plants grown in high saline soil (EC ≥ 4 dS/m) conditions, with the grain yield unobtainable in the salt-sensitive cultivars at 8 and 12 dS/m of salinity (Hakim *et al*., 2014). Our results revealed that rice yield and yield components at the high-saline soil field were more severely affected by salinity than those at the low-saline soil field, as evidenced by a profound reduction in the number of filled grains per hill (Table 7 and 9). The findings obtained in our study were in good agreement with a previous study which showed that the yields of four rice genotypes planted at the high-saline (EC > 4 dS/m) site were much lower than those at the low-saline (EC < 2 dS/m) site (Saleque *et al*., 2005). Our results also showed in Table 9 that at the high-saline field, the number of filled grains per hill was reduced by 66 and 33% in 'RD6' and 'KDML105', respectively, compared to those in the low saline field (Table 7). Additionally, our results showed that at harvest, 'RD6' was found to show more severe symptoms of leaf yellowing/browning than 'KDML105'. These findings indicated that 'KDML105' had better adaptations to high-severity salinity than 'RD6', which was well supported by previous studies, which elucidated that even though both 'KDML105' and 'RD6' have been considered as salt-sensitive cultivars, KDML105 was found to show better tolerance to salinity (Cha-um *et al*., 2010; Nishimura *et al*., 2011).

At both fields, foliar spraying with 0.5 mM Spd or 1:300 and 1:500 dilutions of wood vinegar substantially improved rice yield and yield components (Tables 7-10). At the low saline soil field, the number of filled grains per hill was increased by 66, 52 and 47% in 'KDML105' and by 25, 2 and 2% in 'RD6' when 0.5 mM Spd, and 1:300 and 1:500 dilutions of wood vinegar were applied, respectively (Table 7). The findings obtained in our study were in agreement with a previous study which reported that foliar spraying of salt-stressed rice plants at the booting stage with 1.0 mM Spd increased the yield of rice plants (Saleethong *et al*., 2013). At the high saline soil field, the yield was increased by 37, 18 and 49% in 'KDML 105' and by 2, 36 and 45% in 'RD6' when 0.5 mM Spd, and 1:300 and 1:500 dilutions of wood vinegar were applied, respectively (Table 9). Our findings suggested that wood vinegar applied at a dilution of 1:500 was the most appropriate for improving the yield of both cultivars under high saline soil field conditions, followed by a dilution of 1:300. Our result is in agreement with previous studies of Kulkarni *et al*., (2006) which demonstrated that plant-derived smoke-water containing butenolide was the most effective at a dilution of 1:500 in improving the growth of rice seedlings. In addition, *Bauhinia*-derived smoke solution applied at a dilution of 1:500 was the most effective in alleviating salt stress in the indica rice at both physiological and biochemical levels (Jamil *et al*., 2014). Our findings suggested that foliar application of Spd and/or wood vinegar can be used for improving rice yield and yield components under both low and high saline soil field conditions.

Table 5. Effects of foliar sprays of Spd (0, 0.1, 0.5 and 1 mM) on number of filled grains per pot, filled grain weight per pot, root weight, straw weight, panicle length, aboveground weight, biomass and harvest index of 'KDML105' rice plants observed at the end of salt stress treatment.

Treatment	No. filled grains/ pot	Filled grain weight/ pot (g)	Root weight (g)	Straw weight (g)	Panicle weight (g)	Aboveground weight (g)	Biomass (g)	Harvest index (%)
Control	500.84bc	12.98bc	12.07c	19.26bc	13.89a	40.84ab	52.91ab	19.85a
NaCl	442.06c	10.41d	13.49bc	23.52a	10.97bc	41.19ab	54.69ab	14.48c
0.1 mM Spd	532.28b	14.10b	11.18c	19.86a-c	11.58a-c	39.28ab	50.47b	20.49a
0.1 mM Spd + NaCl	445.09c	10.45d	12.14c	21.10a-c	12.10ab	39.07ab	51.20b	15.15bc
0.5 mM Spd	663.63a	17.27a	17.01ab	20.96a-c	12.36ab	42.75a	59.76a	20.28a
0.5 mM Spd + NaCl	707.94a	17.28a	18.43a	22.94ab	10.12bc	41.34ab	59.76a	16.93a-c
1 mM Spd	478.84bc	12.42c	14.08bc	17.79c	10.35ab	36.63b	50.71b	21.18a
1 mM Spd + NaCl	452.50bc	11.73cd	16.62ab	19.96a-c	9.41c	37.78ab	54.40ab	19.30ab

*Means in the same column followed by different letters are statistically different at $p<0.05$

Table 6. Effects of foliar sprays of Spd (0, 0.1, 0.5 and 1 mM) on seed width and length, seed length/width ratio, grain width, grain length, and grain length/width ratio of 'KDML105' rice plants observed at the end of salt stress treatment.

Treatment	Seed width (mm)	Seed length (mm)	Seed length/width ratio	Grain width (mm)	Grain length (mm)	Grain length/ width ratio
Control	2.20a	7.56a	3.46a	2.39ab	10.57a-c	4.43
NaCl	2.08ab	6.66c	3.21b	2.31b	10.20c	4.44
0.1 mM Spd	2.09ab	7.65a	3.65a	2.64a	10.67ab	4.17
0.1 mM Spd + NaCl	2.16ab	6.89b	3.21b	2.45ab	10.25c	4.21
0.5 mM Spd	2.05ab	7.45a	3.64a	2.48ab	10.59a-c	4.28
0.5 mM Spd + NaCl	2.11b	6.83bc	3.24b	2.53ab	10.69a	4.23
1 mM Spd	2.09ab	7.53a	3.60a	2.43ab	10.28bc	4.27
1 mM Spd + NaCl	2.13ab	6.88b	3.24b	2.51ab	10.37a-c	4.15

*Means in the same column followed by different letters are statistically different at $p<0.05$

Table 7. Effect of foliar sprays of 0.5 mM Spd and wood vinegar (1:300 and 1:500 dilutions) on number of filled grains per panicle, number of unfilled grains per panicle, number of total grains per panicle, percent seed setting, percent unfilled grains, 1000-grain weight, filled grain weight per panicle, panicle weight, number of filled grains per hill and filled grain weight per hill of 'KDML105' and 'RD6' rice plants grown under low saline soil conditions with an EC of 3 dS/m and an alkaline pH of 8.0.

Treatment	KDML 105									
	No. of filled grains/ panicle	No. unfilled grains/ panicle	No. of total grains/ panicle	Seed setting (%)	Unfilled grain (%)	1000-grain weight (g)	Filled grain weight/ panicle	Panicle weight (g)	No. of filled grains/ hill	Filled grain weight/ hill (g)
Control	114.36b	31.59a	145.95b	78.26c	21.74a	23.21b	3.02b	3.35b	1027.84c	23.83b
0.5 mM Spd	137.31a	24.03b	161.34a	85.07ab	14.93ab	24.71a	3.41a	3.68ab	1709.05a	42.25a
1:300 wood vinegar	120.66b	26.30b	146.96b	82.03b	17.97b	25.70a	3.27ab	3.50ab	1563.81b	40.21a
1:500 wood vinegar	136.19a	22.12b	158.31ab	86.02a	13.99c	25.45a	3.59a	3.87a	1514.40b	38.56a
Treatment	RD6									
	No. of filled grains/ panicle	No. unfilled grains/ panicle	No. of total grains/ panicle	Seed setting (%)	Unfilled grain (%)	1000-grain weight (g)	Filled grain weight/ panicle	Panicle weight (g)	No. of filled grains/ hill	Filled grain weight/ hill (g)
Control	174.01	28.88a	202.88	85.84b	14.16a	24.02b	4.29b	4.53b	1994.81b	47.95b
0.5 mM Spd	194.30	22.26bc	216.56	89.68a	10.33b	24.51ab	5.06a	5.30a	2494.72a	61.17a
1:300 wood vinegar	178.38	20.47c	198.85	89.64a	10.36b	24.23b	4.34b	4.58b	2037.65b	49.34b
1:500 wood vinegar	173.68	27.23ab	200.90	86.42b	13.59a	24.94a	4.22b	4.52b	2029.69b	50.64b

***** Means in the same column followed by different letters are statistically different at $p<0.05$

Table 9. Effect of foliar sprays of 0.5 mM Spd and wood vinegar (1:300 and 1:500 dilutions) on number of filled grains per panicle, number of unfilled grains per panicle, number of total grains per panicle, percent seed setting, percent unfilled grains, 1000-grain weight, filled grain weight per panicle, panicle weight, number of filled grains per hill and filled grain weight per hill of 'KDML 105' and 'RD6' rice plants grown under high saline soil conditions with an EC of 10 dS/m and an alkaline pH of 8.2.

Treatment	KDML 105									
	No. of filled grains/ panicle	No. unfilled grains/ panicle	No. of total grains/ panicle	Seed setting (%)	Unfilled grain (%)	1000-grain weight (g)	Filled grain weight/ panicle	Panicle weight (g)	No. of filled grains/ hill	Filled grain weight/ hill (g)
Control	68.34b	32.24a	100.58	67.81d	32.19a	22.84b	1.70	5.12a	693.61c	15.86b
0.5 mM Spd	75.17b	21.15c	96.32	77.91b	22.09c	24.99a	1.78	3.98b	952.48ab	23.81a
1:300 wood vinegar	71.16b	25.23b	96.38	73.78c	26.22b	23.72ab	1.75	4.37b	815.96bc	19.38b
1:500 wood vinegar	86.90a	20.23c	107.13	81.16a	18.84d	24.32ab	1.97	3.86b	1029.78a	25.00a
Treatment	RD6									
	No. of filled grains/ panicle	No. unfilled grains/ panicle	No. of total grains/ panicle	Seed setting (%)	Unfilled grain (%)	1000-grain weight (g)	Filled grain weight/ panicle	Panicle weight (g)	No. of filled grains/ hill	Filled grain weight/ hill (g)
Control	74.55b	26.50	101.05b	73.87	26.13ab	23.57	1.76b	4.45b	681.63b	16.06b
0.5 mM Spd	71.62b	27.53	99.14b	72.15	27.86a	23.48	1.84b	4.64b	697.39b	16.39b
1:300 wood vinegar	92.95a	24.50	118.85a	78.18	20.63b	24.14	2.25ab	4.34b	923.57a	22.29a
1:500 wood vinegar	88.74a	28.25	117.85a	75.34	23.91ab	24.62	2.46a	5.02a	985.75a	24.25a

***** Means in the same column followed by different letters are statistically different at $p<0.05$

Table 8. Effect of foliar sprays of 0.5 mM Spd and wood vinegar (1:300 and 1:500 dilutions) on plant height, number of tillers per hill, number of panicles per hill, straw weight, harvest index and biomass of 'KDML105' and 'RD6' rice plants grown under low saline soil conditions with an EC of 3 dS/m and an alkaline pH of 8.0.

Treatment	KDML 105					
	Plant height (cm)	No. of tillers/ hill	No. of panicles/ hill	Straw weight (g)	Harvest index (%)	Biomass (g)
Control	136.05b	9.35b	9.35b	107.81c	20.02b	134.79c
0.5 mM Spd	140.55ab	12.20a	12.70a	134.58a	25.43a	180.41a
1:300 wood vinegar	145.35a	11.85a	11.95a	132.37a	24.53a	175.37a
1:500 wood vinegar	144.10ab	11.95a	11.45b	119.54b	25.94a	161.36b
Treatment	**RD6**					
	Plant height (cm)	**No. of tillers/ hill**	**No. of panicles/ hill**	**Straw weight (g)**	**Harvest index (%)**	**Biomass (g)**
Control	141.85	11.90	11.10a	114.31c	29.89	164.85b
0.5 mM Spd	140.50	11.70	9.65ab	129.99a	32.76	193.42a
1:300 wood vinegar	137.30	11.00	8.45b	117.48bc	30.33	168.80ab
1:500 wood vinegar	143.35	10.95	9.90ab	125.90ab	29.81	179.48b

* Means in the same column followed by different letters are statistically different at $p<0.05$

Table 10. Effect of foliar sprays of 0.5 mM Spd and wood vinegar (1:300 and 1:500 dilutions) on plant height, number of tillers per hill, number of panicles per hill, straw weight, harvest index and biomass of 'KDML105' and 'RD6' rice plants grown under high saline soil conditions with an EC of 10 dS/m and an alkaline pH of 8.2.

Treatment	KDML 105					
	Plant height (cm)	No. of tillers/ hill	No. of panicles/ hill	Straw weight (g)	Harvest index (%)	Biomass (g)
Control	103.20	11.80a	10.65	51.87	50.15	100.70b
0.5 mM Spd	108.45	12.70a	11.90	57.63	46.58	110.79a
1:300 wood vinegar	105.65	11.45ab	10.55	51.28	48.09	101.81b
1:500 wood vinegar	107.95	10.45b	11.80	49.45	48.93	99.38b
Treatment	**RD6**					
	Plant height (cm)	**No. of tillers/ hill**	**No. of panicles/ hill**	**Straw weight (g)**	**Harvest index (%)**	**Biomass (g)**
Control	107.80	9.15b	8.30b	48.43b	44.30ab	86.99b
0.5 mM Spd	105.30	9.30b	9.15b	43.27b	48.93a	84.33b
1:300 wood vinegar	107.45	9.95ab	9.45ab	50.26b	45.50ab	92.03b
1:500 wood vinegar	110.25	10.90a	10.60a	70.21a	41.56b	119.92a

* Means in the same column followed by different letters are statistically different at $p<0.05$

Conclusions

This study has highlighted the beneficial effects of exogenous Spd and wood vinegar on growth and yield of two rice cultivars ('KDML105' and 'RD6') under saline soil conditions. Our results showed that 0.5 mM Spd was the most effective in improving the growth and yield of the studied rice cultivars under both greenhouse and field conditions. In the low saline soil field, 0.5 mM Spd was the most effective in alleviating salinity stress in 'KDML105'. Meanwhile, in the high saline soil field, wood vinegar at a dilution of 1:500 was the most effective in both cultivars. Our findings suggested that wood vinegar can be used as an alternative for foliar-spraying to improve rice growth and yield under saline soil field conditions since it is inexpensive, readily available and easily accessible on the local market.

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References

Abdallah, M.M.S., Z.A. Abdelgawad and H.M.S. El-Bassiouny. 2016. Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. *S. Afr. J. Bot.*, 103: 275-282.

Aldesuquy, H., Z. Baka and B. Mickky. 2014. Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. *Egypt. J. Basic Appl. Sci.*, 1: 77-87.

Bimpong, I.K., B. Manneh, M. Sock, F. Diaw, N.K.A. Amoah, A.M. Ismail, G. Gregorio, R.K. Singh and M. Wopereis. 2016. Improving salt tolerance of lowland rice cultivar 'Rassi' through marker-aided backcross breeding in West Africa. *Plant Sci.*, 242: 288-299.

Chattopadhayay, M.K., B.S. Tiwari, G. Chattopadhyay, A. Bose, D.N. Sengupta and B. Ghosh. 2002. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. *Physiol. Plant.*, 116: 192-199.

Cha-um, S., N.T.H. Nhung and C. Kirdmanee. 2010. Effect of mannitol- and salt-induced iso-osmotic stress on proline accumulation, photosynthetic abilities and growth characters of rice cultivars (*Oryza sativa* L. spp. *indica*). *Pak. J. Bot.*, 42: 927-941.

Chunthaburee, S., J. Sanitchon, W. Pattanagul and P. Theerakulpisut. 2015a. Application of exogenous spermidine (Spd) improved salt tolerance of rice at the seedling and reproductive stages. *Procedia Environ. Sci.*, 29: 134.

Chunthaburee, S., J. Sanitchon, W. Pattanagul and P. Theerakulpisut. 2015b. Effects of salt stress after late booting stage on yield and antioxidant capacity in pigmented rice grains and alleviation of the salt-induced yield reduction by exogenous spermidine. *Plant Prod. Sci.*, 18: 32-42.

Dissatian, A., J. Sanitchon, P. Pongdontri, N. Jongrungklang and D. Jothityangkoon. 2018. Potential of wood vinegar for enhancing seed germination of three upland rice varieties by suppressing malondialdehyde production. *AGRIVITA J. Agr. Sci.*, 40(2): 371-380.

Djanaguiraman, M., J.A. Sheeba, A.K. Shanker, D.D. Devi and U. Bangarusamy. 2006. Rice can acclimate to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. *Plant Soil.*, 284: 363-373.

Du, C.X., H.F. Fan, S.R. Guo and T. Tezuka. 2010. Applying spermidine for differential responses of antioxidant enzymes in cucumber subjected to short-term salinity. *J. Amer. Soc. Hort. Sci.*, 135(1): 18-24.

Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot.*, 55(396): 307-319.

Ghosh, N., M.K. Adak, P.D. Ghosh, S. Gupta, D.N. Sen Gupta and C. Mandal. 2011. Differential responses of two rice varieties to salt stress. *Plant Biotechnol. Rep.*, 5(1): 89-103.

Grattan, S.R., L. Zeng, M.C. Shannon and S.R. Roberts. 2002. Rice is more sensitive to salinity than previously thought. *Calif. Agric.*, 56: 189-198.

Hakim, M.A., A.S. Juraimi, M.M. Hanafi, M.R. Ismail, A. Selamat, M.Y. Rafii and M.A. Latif. 2014. Biochemical and anatomical changes and yield reduction in rice (*Oryza sativa* L.) under varied salinity regimes. *Biol. Med. Res. Int.*, 208584.

Hoang, T.M.L., L. Moghaddam, B.Williams, H. Khanna, J. Dale and S.G. Mundree. 2015. Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. *Front. Plant Sci.*, 6: 175.

Jamil, M., M. Kanwal, M.M. Aslam, S.U. Khan, I. Malook, J. Tu and S. ur Rehman. 2014. Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. *Aust. J. Crop Sci.*, 8: 159-170.

Kaur, N., K. Kirat, S.Saini, I. Sharma, P. Gantet and P.K. Pati. 2016. Reactive oxygen species generating system and brassinosteroids are linked to salt stress adaptation mechanisms in rice. *Plant Signal. Behav.*, 11(12): e1247136.

Kulkarni, M.G., S.G. Sparg, M.E. Light and J. Van Staden. 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *J. Agron. Crop Sci.,* 192(5): 395-398.

Liu, M., M. Chu, Y. Ding, S. Wang, Z. Liu, S. Tang, C. Ding and G. Li. 2015. Exogenous spermidine alleviates oxidative damage and reduce yield loss in rice submerged at tillering stage. *Front. Plant Sci.*, 6: 919.

Mungkunkamchao, T., T. Kesmala, S. Pimratch, B. Toomsan and D. Jothityangkoon. 2013. Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (*Solanum lycopersicum* L.). *Sci. Hort.*, 154: 66-72.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol.*, 167: 645-663.

Munns, R., R.A. James and A.Läuchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57(5): 1025-1043.

Ndayiragije, A. and S. Lutts. 2007. Long term exogenous putrescine application improves grain yield of a salt-sensitive rice cultivar exposed to NaCl. *Plant Soil.*, 291: 225-238.

Nishimura, T., S. Cha-um, M. Takagaki, K. Ohyama and C. Kirdmanee. 2011. Survival percentage, photosynthetic abilities and growth charactersof two indica rice (*Oryza sativa* L. spp. *indica*) cultivars in responseto iso-osmotic stress. *Span. J. Agric. Res.*, 9: 262-270.

Puyang, X., M. An, L. Xu, L. Han and X. Zhang. 2016. Protective effect of exogenous spermidine on ion and polyamine metabolism in Kentucky bluegrass under salinity stress. *Hort. Environ. Biotechnol.*, 57: 11-19.

Reddy, I.N.B.L., B.K. Kim, I.S. Yoon, K.H. Kim and T.R. Kwon. 2017. Salt tolerance in rice: focus on mechanisms and approaches. *Rice Sci.*, 24: 123-144.

Roychoudhury, A., S. Basu and D.N. Sengupta. 2011. Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. *J. Plant Physiol.*, 168: 317-328.

Saha, J. and K. Giri. 2017. Molecular phylogenomic study and the role of exogenous spermidine in the metabolic adjustment of endogenous polyamine in two rice cultivars under salt stress. *Gene*, 609: 88-103.

Saleethong, P., J. Sanitchon, K. Kong-ngern and P. Theerakulpisut. 2013. Effects of exogenous spermidine (Spd) on yield, yield-related parameters and mineral composition of rice (*Oryza sativa* L. ssp. *indica*) grains under salt stress. *Aust. J. Crop Sci.*, 7: 1293-1301.

Saleethong, P., S. Roytrakul, K. Kong-Ngern and P. Theerakulpisut. 2016. Differential proteins expressed in rice leaves and grains in response to salinity and exogenous spermidine treatments. *Rice Sci.*, 23: 9-21.

Saleque, M.A., N.N. Choudhury, S.M. Rezaul Karim and G.M. Panaullah. 2005. Mineral nutrition and yield of four rice genotypes in the farmers' fields of salt-affected soils. *J. Plant Nutr.*, 28: 865-875.

Shereen, A., Asma, M.U. Shirazi, M. Arif, W. Mahboob and M.A. Khan. 2020. Saninity-induced variability in morpho-physiological traits and their relationship with grain yield in rice (*Oryza sativa* L.). *Pak. J. Bot.*, 52(5): 1615-1623.

Suriya-arunroj, D., N. Supapoj, A. Vanavichit and T. Toojinda. 2005. Screening and selection for physiological characters contributing to salinity tolerance in rice. *Kasetsart J.* (*Nat. Sci.*), 39: 174-185.

Taïbi, K., F. Taïbi, L. Ait Abderrahim, A. Ennajah, M. Belkhodja and J.M. Mulet. 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *S. Afr. J. Bot.*, 105: 306-312.

Theerakulpisut, P., N. Kanawapee and B. Panwong. 2016. Seed priming alleviated salt stress effects on rice seedlings by improving Na^+/K^+ and maintaining membrane integrity. *Int. J. Plant Bio.*, 7: 53-58.

Thitisaksakul, M., K. Tananuwong, C.F. Shoemaker, A. Chun, O.U. Tanadul, J.M. Labavitch and D.M. Beckles. 2015. Effects of timing and severity of salinity stress on rice (*Oryza sativa* L.) yield, grain composition, and starch functionality. *J. Agric. Food Chem.*, 63: 2296-2304.

Wankhade, S.D., M.J. Cornejo, I. Mateu-Andrés and A. Sanz. 2013. Morpho-physiological variations in response to NaCl stress during vegetative and reproductive development of rice. *Acta Physiol. Plant.*, 35: 323-333.

Wu, Q., S. Zhang, B. Hou, H. Zheng, W. Deng, D. Liu and W. Tang. 2015. Study on the preparation of wood vinegar from biomass residues by carbonization process. *Biores. Technol.*, 179: 98-103.

Yuvaniyama A. 2004. *Management of Saline Soil in Northern Part of Thailand*. Land Development Department, Bangkok.

Zeng, Y.H., Y.P. Zahng, J. Xiang, H. Wu, H.Z. Chen, Y.K. Zhang and D.F. Zhu. 2016. Effects of chilling tolerance induced by spermidine pretreatment on antioxidative activity, endogenous hormones and ultrastructure of indica-japonica hybrid rice seedlings. *J. Integr. Agri.*, 15: 295-308.

Zhang, H., W. Liang, X. Yang, X. Luo, N. Jiang, H. Ma and D. Zhang. 2010. Carbon starved anther encodes a MYB domain protein that regulates sugar partitioning required for rice pollen development. *Plant Cell,* 22: 672-689.

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