

Effects of exogenous spermidine (Spd) on yield, yield-related parameters and mineral composition of rice (*Oryza sativa* L. ssp. *indica*) grains under salt stress**Paweena Saleethong¹, Jirawat Sanitchon², Kanlaya Kong-ngern¹ and Piyada Theerakulpisut^{1*}**¹Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand²Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

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Abstract

Effects of spermidine (Spd) on yield, yield-related parameters and mineral composition in mature grains of rice exposed to NaCl stress were investigated in rice cultivars Pokkali (salt-tolerant) and KDML105 (salt-sensitive). Plants were grown in pots until early booting stage after which the plants were sprayed with 1 mM Spd for 7 successive days before being stressed by irrigating with 25 mM NaCl instead of tap water until maturity. Salt stress resulted in 32% and 56% reduction in grain yield per plant in Pokkali and KDML105 respectively. Pretreatment with Spd dramatically improved grain yield per plant of KDML105 from 17.7 to 28.7 g (62% increase) and Pokkali from 31.0 to 36.2 g (16% increase). Salt stress resulted in significant reductions in macronutrient elements i.e. N, P, K and Mg but an increase in Ca in brown rice grains of both cultivars. Although Spd pretreatment did not increase the salt-inhibited accumulation of N, P and Mg, it did improve K content and inhibited Na uptake leading to a large improvement in K/Na ratio. Spd pretreatment generally did not have any beneficial effects on the composition of micronutrient elements. Spd pretreatment considerably improved the red pigmentation in pericarp of salt-treated Pokkali grains and slightly increased 2-acetyl-1-pyrroline (2-AP) in KDML105 grains. The present study demonstrated that exogenous Spd application alleviated the adverse effects of NaCl stress on the reproductive processes leading to an improvement in rice yield, particularly in the salt-sensitive cultivar. However, to exploit Spd for agricultural benefits more research is needed to evaluate the most suitable plant developmental stages as well as the most cost-effective concentrations and methods of Spd application.

Keywords: mineral composition; rice; salt stress; spermidine; yield.**Abbreviations:** 2-AP_ 2-acetyl-1-pyrroline; KDML105_Khaw Dawk Mali 105; PAs_ polyamines; Put_putrescine; Spd_spermidine; Spm_spermine.**Introduction**

Salinity is an increasingly important abiotic stress factor limiting growth and yield of crop plants in arid and semi-arid regions of the world. Rice is more sensitive to salt stress compared with other cereals (Shannon et al., 1998) and the threshold above which rice yields reduced ranges from 1.9 to 3 dS m⁻¹ (Grattan et al., 2002). Salinity inhibits plant growth primarily because it causes osmotic stress and ion toxicity, and secondarily it leads to nutritional deficiency and oxidative stress (Zhu, 2002). Salinity affects all stages of growth and development of rice plants. Rice is relatively salt tolerant during germination, active tillering, and towards maturity, but sensitive during the early seedling and reproductive stages (Flowers and Yeo, 1981; Lutts et al., 1995). The physiological bases of salt tolerance during the vegetative stage includes: salt exclusion or low ion uptake; partitioning of toxic ions to older tissues; higher tissue tolerance by partitioning salts into vacuoles; vigorous growth to dilute salt concentrations; and, responsive stomata and efficient antioxidant systems (Yeo and Flowers, 1986; Ismail et al., 2007; Türkan and Demiral, 2009). During reproductive development, tolerant genotypes tend to exclude salt from flag leaves and developing panicles (Yeo and Flower, 1986; Moradi et al., 2003). Various approaches have been made to develop high-yielding salt tolerance rice cultivars by (1) marker-assisted breeding to pyramid new genes determining resistant physiological traits from donor germplasm; and, (2)

transformation with single genes and *in vitro* approaches including somaclonal variations and anther culture (Gregorio et al., 2002; Munns et al., 2006). Considerable progress has been made but commercial success has been limited owing to the complexity of traits involved in tolerance of salt stress, persistence of undesirable traits from donor germplasm, and coexistence of multiple abiotic stresses under field conditions (Ismail et al., 2007). Recently, as reviewed by Hamdia and Shaddad (2010), several groups of researchers has been using alternative approaches to try to improve plant tolerance to salinity and yield through either chemical treatments (plant hormones, minerals, compatible solutes, vitamins and polyamines) or biofertilizers. Polyamines (PAs) are low molecular weight, non-protein, straight chain, aliphatic hydrocarbon compounds with amino and imino groups that include putrescine (Put), spermidine (Spd) and spermine (Spm). They are involved in a wide range of biological processes because of their cationic nature that assists in their interaction with DNA, protein, membrane phospholipids, and cell wall polysaccharides (Lui et al., 2007). Accumulation of polyamines in response to stress has been reported to be associated with abiotic stress resistance in several plant species (Bouchereau et al., 1999). Numerous reports have presented evidence that exogenous polyamine application increased endogenous PAs and offered protection and alleviation of salt stress damage in vegetative tissues of several plants including rice (Krishnamurthy and Bhagwat,

1989; Chattopadhyay et al., 2002; Verma and Mishra, 2005; Alcázar et al., 2006; Duan et al., 2008; Roychoudhury et al., 2011; Saleethong et al., 2011). Exogenous application of PAs has also been reported to promote reproductive development under normal growth conditions and offer protection to reproductive structures against abiotic stress (Nayyar, 2005; Malik and Singh, 2006). An improvement of floral morphogenesis was observed in salt-treated rice plants exposed to Put leading to a higher number of fertile tiller per plant, higher number of spikelets per panicle and higher grain yield per plant (Ndayiragije and Lutts, 2007). Effects of exogenous PAs on alleviation of salt stress and their physiological mechanisms have been elucidated mainly from studies in vegetative tissues of a few model plants including rice. To our knowledge, little evidence exists on the effects of exogenous Spd application on reproductive development and yield of rice exposed to salt stress. The objectives of this study were to investigate the effects of exogenous Spd application on yield and yield-related parameters of rice, and also on grain quality in terms of the composition of mineral nutrients. Two rice cultivars were compared, namely Pokkali and Khao Dawk Mali 105 (KDML105). Pokkali is a salt-tolerant landrace variety cultivated in coastal Southern India often used as a donor for salt tolerant alleles in breeding programs. It consists of deep red pericarp composing the health-promoting pigments proanthocyanidins. Khao Dawk Mali 105 (KDML105) is a salt-sensitive economically important white rice widely known for its good cooking quality and distinctive fragrance contributed by the volatile compound 2-acetyl-1-pyrroline (2-AP). The positive impact of Spd on the enhancement of crop yield and quality is expected to form a basis for more active research for a deeper understanding of the mechanisms of action of PAs in modulating the reproductive development, and for a future investigation to optimize the use of PAs as a plant growth regulator for crop production enhancement.

Results

Effect of exogenous spermidine on yield and yield components of rice exposed to salt stress

Rice plants exposed to salt stress from the booting stage through to maturity showed a significant reduction in yield and yield-related parameters. Salinity did not affect the number of panicles per plant in Pokkali but dramatically reduced this parameter in KDML105 (Fig. 1a). Conversely, the reduction in panicle length (Fig. 1b) and the number of primary branches (Fig. 1c) were more pronounced in Pokkali than KDML105. Salinity resulted in a reduction in the number of filled grains per panicle (Fig. 1d), an increase in the number of unfilled grains per panicle (Fig. 1e), which was the consequence of the reduction of panicle fertility (Fig. 1f). The changes in filled grain weight/panicle (Fig. 1g) and unfilled grain weight /panicle (Fig. 1h) followed similar patterns as those of filled and unfilled grain numbers. The effects of salt stress on the reduction in 1000-grain weights (Fig. 1i) were less severe than those on the number of filled grains per panicle. The most pronounced effect of salt stress was demonstrated in the grain yield per plant (Fig. 1j). Grain yield per plant for Pokkali fell from 45.5 g in the control to 31 g in the salt-stressed group (32% reduction), and for KDML105 from 40.5 g to 17.7 g (56% reduction). Pretreatment of Spd was accomplished by foliar spraying for 7 days while the panicles were developing inside the flag leaf sheath. This significantly alleviated the adverse effects of salt stress on all aspects of yield-related characters (except the

number of primary branches). The number of panicles in KDML105 increased from 6 in the salt-treated plants to 8.4 in the Spd-pretreated group (33% increase). The length of panicles was slightly but significantly increased in both cultivars (8% and 5% increase in Pokkali and KDML105, respectively). The pronounced effect of Spd was observed in the improvement of panicle fertility. Panicle fertility of KDML105 was raised from 73.15% to 85% (16% increase) which was higher than that in the control (81.69%). For Pokkali, panicle fertility increased from 80.9% to 86.2% (6% increase). Consequently, the number of filled grains per panicle increased from 92.98 to 107.7 (16% increase) in Pokkali, and from 85.9 to 102.46 (20% increase) in KDML105. The 1000-grain weight increased only slightly for both cultivars. Finally, grain yield per plant dramatically increased in KDML105 from 17.7 to 28.7 g (62% increase) and from 31 to 36.2 g (16% increase) in Pokkali.

Effect of exogenous spermidine on mineral composition in mature rice grains

The mineral contents in Pokkali's grains harvested from plants grown under non-stressed conditions were generally higher than those in the KDML105 grains (Table 1). The differences were most pronounced in three of the four microelements investigated i.e. the concentrations of Mn, Cu and Zn were more than three folds higher in Pokkali. When the plants were stressed with NaCl, the concentrations of most elements differed significantly from those in the control. As shown in Table 1, the concentrations of N, P, K and Mg in the mature grains decreased, while those of Na and Cu increased for both cultivars. Salt affected the concentrations differently for Fe and Zn, i.e. they increased in KDML105 but decreased in Pokkali. Salt stress did not have any effects on the concentrations of Ca and Mn in KDML105 grains but caused a significant increase and decrease in Ca and Mn, respectively for Pokkali. Treatment with Spd without salt stress, in most cases, did not affect mineral contents in KDML105 grains, except for some significant increases in Na, Cu and Zn. Spd tended to have more negative effects on the mineral contents of Pokkali grains, causing significant reductions in N, Mg, Mn, Cu and Zn, with the exception of K which increased significantly. When the plants were sprayed with Spd prior to salt stress, K concentration significantly increased up to the same level as the controls in both cultivars whereas Na significantly decreased in KDML105 only. As a result of Spd treatment, the level of Na in salt stressed KDML105 grains decreased dramatically down to the similar level as the control. Spd had an opposite effect on Fe accumulation of the two cultivars, i.e. it increased in Pokkali but decreased in KDML105. Spd had no effects on the concentration of Mn, Cu and Zn in KDML105 but resulted in a significant reduction of these elements in Pokkali. However, Spd did not have any significant effects on the concentrations of P and Ca in both cultivars.

Effect of exogenous spermidine on pericarp color and 2-AP in mature rice grains

As shown in Fig. 2a, mature rice grains harvested from Pokkali rice plants treated with Spd (without NaCl) and NaCl stress showed a dramatic reduction in the intensity of pericarp color compared with rice grains from control plants. Pretreatment with Spd considerably increased the color intensity of the salt-stressed rice grains. However, the pericarp color of KDML105 grains was insensitive to neither

Spd nor NaCl treatment. The 2-AP content in mature brown rice grains of KDML105 significantly decreased when the plants were exposed to both NaCl and Spd (without NaCl). When the plants were treated with Spd before being exposed to salt stress, 2-AP content increased slightly but not statistically different from the salt-treatment (Fig. 2b).

Discussion

Salinity which was initiated at the late booting stage and continued through to seed maturity resulted in greater reduction in grain yield per plant in KDML105 (56% reduction) than in Pokkali (32% reduction). All yield components measured were significantly reduced in KDML105. However, the most important contributory factor leading to marked yield reduction in this cultivar were the reduction in the panicle number per plant (48% reduction), and the second most was the reduction in filled grains per panicle (20% reduction). Filled grains per panicle and panicle number per plant have been shown to be the major contributors to salinity induced yield loss (Asch et al., 1999; Zeng et al., 2002; Shereen et al., 2005; Mahmood et al., 2009). The most important components contributing to yield loss of Pokkali were the reduction in fertility (10%) leading to the reduced number of filled grains (27% reduction) and increased number of unfilled grains per panicle (55% increase). Comparing yield and yield components under salinity between Pokkali and seven less tolerant plus one more tolerant improved (line NR-2) genotypes, Ali et al. (2004) also found that the number of panicles per plant of Pokkali was less affected compared to the less tolerant lines. Pokkali, on the other hand, showed relatively high reduction in panicle fertility (22.1%) only slightly lower than that in the most sensitive genotype (Basmati-370; 25.3% reduction). Compared to grain number, grain weight expressed as 1000-grain weight was much less affected by salinity (10% and 4% reduction in Pokkali and KDML105 respectively). In the study comparing yield and yield components of 12 rice genotypes under salinity stress, Zeng et al. (2002) also found that grain weight was much less sensitive to salinity, and an extremely low correlation (0.01) was detected between seed yield and seed weight. Reduction in panicle number was the most important factor which caused the higher percentage of reduction in seed yield per plant in KDML105 than in Pokkali. Six closely related rice inbred lines exposed to 50 mM NaCl (from 7-week old stage through to maturity) also showed extremely wide variations in changes in panicle number from 5-52% reduction to 10% increase (Shereen et al., 2005). Similarly, Ali et al. (2004) found that the reduction in panicle number varied among ten rice cultivars from 4.78% to 28.1% when the plants were exposed to salinity from 40-week old stage to maturity. In contrast, when rice plants were exposed to salinity at the early booting stage, and then the stress was relieved after 20 days, grain weight per plant was slightly reduced but the panicle number was unaffected (Zeng et al., 2001). The same authors found that a 20-day salinity period imposed during the vegetative growth stage significantly reduced seed yield and most yield components. Thus, yield of rice under salinity stress depended on both plant growth stage, and duration and intensity of the stress (Shalhevet, 1994; Zeng et al., 2001). The present study demonstrated that exogenous application of Spd during the panicle development stages reduced the deleterious effects of NaCl stress on rice yield in both salt-tolerant and salt-sensitive genotypes. Almost all yield-related characters (except the number of primary branches in the panicle) which were negatively affected by salt stress were

alleviated in the stressed plants pretreated with Spd. The major yield components which were improved and contributed most to a dramatic increase in seed yield of KDML105 (62% increase) were the panicle number, filled grains per panicle and panicle fertility. The most improved contributory factors for Pokkali were filled grains per panicle, and panicle fertility. Spd therefore effectively stimulated the development of reproductive structures as well as the post-anthesis development. Similar results were obtained by Ndayiragije and Lutts (2007) who demonstrated that exogenous Put, and to a lesser extent Spd, improved salt-damage yield-related characters in salt-sensitive rice (cv. I Kong Pao) including grain yield per plant, panicle number, spikelets per panicle, seed set, and 1000-grain weight. Stimulation of reproductive development by PAs has also been reported in several other plants, mostly dicots (Tareghi and Martin-Tanguy, 1995; Albuquerque et al., 2006; Bibi et al., 2010) but the mechanisms of action of PAs on promoting reproductive development are still largely unknown. Data on the positive effects of PAs on reproductive development of rice and other cereal plants are extremely scarce. Ndayiragije and Lutts (2007) found that salt-alleviating effects of exogenous Put on rice plants occurred during floral morphogenesis (increased fertile tillers and spikelets per panicle) as well as post-anthesis development (increased percent pollen viability). Similar to the results in this study, the impact of the weight of 1000-grain weight was relatively small and the effects of exogenous PAs on the improvement of this parameter was insignificant in the study of Ndayiragije and Lutts (2007) and were significant but relatively small in this study (3% increase for both cultivars). However, Anbazhagan et al. (1987) found that foliar spraying of PAs during panicle development resulted in a higher grain filling rate and final grain weight per panicle. Moreover, Spd was found to be more effective than Put and Spm. Most interestingly, Tan et al. (2009) found in six rice cultivars that the grain filling rate and brown rice weight were highly correlated with endogenous Spd and Spm concentrations in developing grains. Moreover, application of exogenous Spd and Spm at the early grain filling stage significantly enhanced activity of starch-synthesizing enzymes and increased grain filling rate, seed setting rate and grain weight. It is also interesting to note that the magnitude of improvement was more pronounced in the salt-sensitive KDML105 than the salt-tolerant Pokkali in most parameters. Salinity induces nutritional disorders which may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant (Grattan and Grieve, 1998). Reduced N and P contents of plants at high salinity have been reported in a range of crops such as forage grasses (Suyama et al., 2007), maize (Tuna et al., 2008), and cauliflower and broccoli (De Pascale et al., 2005). Inhibition of nitrogen uptake may occur by $\text{NO}_3^-/\text{Cl}^-$ interaction at the sites for ion transport (Cram, 1983), because sodium results in severe membrane depolarization in plants (Suhayda et al., 1990) which has been linked to non-competitive inhibition of nitrate uptake (Hawkins and Lewis, 1993). Salinity-induced reductions in P concentrations in plant tissues was suggested to be related to the reduction in P availability in saline soils due to ionic-strength effects that reduce the activity of P, and because P concentrations in the soil solution are tightly controlled by absorption processes and by the low solubility of Ca-P minerals (Grattan and Grieve, 1998). Relatively little is known in regards to Mg nutrition as affected by salinity. Salinity-induced reduction in Mg concentrations in leaves has been reported in citrus (Ruiz et al., 1997) and alfalfa (Wang and Han, 2007). In contrast to other macronutrient elements,

Table 1. Mineral composition in dehulled grains of rice cvs. KDML105 and Pokkali treated with Spd (1 mM) without and with NaCl (25 mM) stress.

	KDML 105				Pokkali			
	Control	Spd	NaCl	NaCl+Spd	Control	Spd	NaCl	NaCl+Spd
N (%)	1.44±0.02 ^d	1.29±0.02 ^{de}	1.23±0.02 ^f	1.31±0.02 ^e	2.26±0.02 ^a	2.03±0.02 ^b	2.06±0.02 ^b	1.80±0.05 ^c
P (%)	0.38±0.01 ^{cd}	0.39±0.02 ^{cd}	0.33±0.01 ^{de}	0.31±0.03 ^e	0.51±0.02 ^a	0.47±0.03 ^{ab}	0.44±0.03 ^{bc}	0.38±0.01 ^{cd}
K (%)	0.24±0.01 ^c	0.24±0.00 ^c	0.20±0.00 ^e	0.24±0.00 ^c	0.28±0.00 ^b	0.31±0.00 ^a	0.22±0.01 ^d	0.31±0.01 ^a
Na (%)	0.044±0.01 ^e	0.049±0.02 ^d	0.057±0.01 ^a	0.045±0.01 ^e	0.049±0.01 ^{cd}	0.053±0.01 ^{bc}	0.055±0.01 ^{ab}	0.054±0.02 ^{ab}
Ca (ppm)	70.00±1.47 ^d	74.00±4.02 ^{bcd}	72.75±3.40 ^{cd}	71.25±1.70 ^d	73.25±4.40 ^{cd}	82.75±3.71 ^{abc}	83.67±0.76 ^{ab}	88.75±3.35 ^a
Mg (ppm)	1610±37.30 ^{cd}	1529±30.28 ^{de}	1472±28.81 ^e	1460±37.25 ^e	1977±13.91 ^a	1765±16.59 ^b	1681±19.55 ^c	1594±20.88 ^d
Fe (ppm)	74.4±4.9 ^{bc}	76.8±3.5 ^b	122.6±7.6 ^a	51.0±4.9 ^d	72.8±7.2 ^{bc}	80.2±4.7 ^b	56.8±6.1 ^{cd}	66.0±5.8 ^{bcd}
Mn (ppm)	10.5±0.63 ^c	12.5±0.87 ^c	11.75±0.48 ^c	10.5±0.87 ^c	41.0±3.63 ^a	21.5±0.87 ^b	24.5±1.55 ^b	11.5±0.50 ^c
Cu (ppm)	3.2±0.2 ^d	6.8±0.3 ^c	7.8±0.7 ^c	6.8±0.3 ^c	9.6±0.2 ^b	7.8±0.3 ^c	12±0.3 ^a	9.6±0.2 ^b
Zn (ppm)	29.8±0.5 ^f	32.6±0.2 ^e	33.6±0.8 ^e	34.6±0.8 ^e	66.8±0.8 ^a	54.8±0.3 ^b	48.4±1.2 ^c	44.4±0.8 ^d
K/Na	5.60±0.2 ^a	4.93±0.14 ^b	3.59±0.07 ^c	5.30±0.11 ^{ab}	5.81±0.19 ^a	5.79±0.17 ^a	4.08±0.14 ^c	5.68±0.23 ^a

Ca increased slightly in KDML105 and significantly in Pokkali under salt stress. Similar results have been reported in forage grasses (Suyama et al., 2007). The contrasting observations between Mg and Ca could also be related to competition effects. It was suggested that Ca is strongly competitive with Mg and the binding strength of the highly hydrated Mg is relatively low at the exchange sites in the cell walls and at the binding sites at the plasma membrane (Marschner, 1995). The effects of salt stress on the reduction in K and the increase in Na content in mature rice grains are the same as that typically observed in the vegetative tissues. The K/Na ratio was more adversely affected in KDML105 than in Pokkali. Asch et al. (1999) found that tolerant rice cultivars had lower panicle sodium concentration at all panicle developmental stages than the susceptible ones resulting in higher yield in the former. It was suggested that the amount of Na taken up by the panicle could be determined before flowering (booting and heading stages) by control mechanism of Na uptake through root properties and the subsequent distribution of Na in the vegetative parts, and after flowering through panicle transpiration. While Spd pretreatment did not increase (except the case for N in KDML105) and in many cases exacerbated the salt-retarded uptake of N, P and Mg, it did improve K and inhibit Na uptake leading to a large improvement in K/Na ratio (Table 1). Many studies have reported this beneficial effect of Spd (and other PAs) in vegetative tissues of rice (Chatopadhyay et al., 2002; Roy et al., 2005; Ndayiragije and Lutts, 2006; Roychoudhury et al., 2011; Salethong et al., 2011). This, as far as we know, is the first report on the alleviation of salt-affected yield reduction in relation to an increase in K/Na ratio in mature rice grains. The mechanism of action of Spd may be related to its stimulation of the NaCl-inhibited plasma membrane-bound H⁺-ATPase (Roy et al., 2005) and the reduction of NaCl-induced K efflux through non-selective cation channels (Shabala et al., 2007). The effects of salinity on the concentrations of micronutrient elements in plants are complex and variable depending upon the level of salinity, macronutrients, plant organs and genotypes. Two main reasons may be responsible for these complex patterns (Hu and Schmidhalter, 2001). First, salinity changes the available concentration of these elements in soils due to an increase in the solubility of micronutrients under saline conditions. Second, genotypes of plants vary widely in their ability to

uptake and metabolize micronutrients. Consequently, salinity may increase, decrease or have no effect on the micronutrient concentration in plant shoots (Grattan and Grieve, 1998). Salinity resulted in a significant increase in Cu in both KDML105 and Pokkali. Similar results have been observed in rice (Alpaslan et al., 1998), soybean (Tunçturk et al., 2008) and canola (Tunçturk et al., 2011). Spd pretreatment, however, resulted in a slight reduction in Cu content, but the concentration of Cu in KDML105 grains treated with Spd+NaCl was still more than twofold that of the control. Of all the micronutrient elements in cereal grains, the concentrations of Fe and Zn are of particular interest because Fe and Zn deficiency are afflicting over three billion people worldwide (Cakmak, 2008). Under normal growth condition, the amounts of Fe were similar in Pokkali and KDML105 grains, but Zn concentration in Pokkali doubled that of KDML105. Salinity treatment had the opposite effects on Fe and Zn content of the two rice cultivars. Earlier reports showed that salinity either increased [cauliflower (De Pascale et al., 2005); soybean (Tunçturk et al., 2008)] or decreased [rice & wheat (Alpaslan et al., 1998); maize (Tuna et al., 2008)] Fe concentrations in plant tissues. Salinity increased the Zn concentration in rice and wheat (Alpaslan et al., 1998), but decreased it in maize (Tuna et al., 2008). Spd pretreatment did not seem to alleviate NaCl-inhibited uptake and accumulation of Fe and Zn in Pokkali grains. Moreover it led to a dramatic reduction of Fe concentration in KDML105 grains reducing it down below the control level. Both salinity and Spd had no effects on the Mn concentration in KDML105 grains, but NaCl adversely affected the Mn content in Pokkali and Spd further exacerbated the effects of NaCl. Some studies have found that salinity reduces the Mn concentrations in plants [maize (Tuna et al., 2008)], while others have reported an increase in Mn concentrations [rice & wheat (Alpaslan et al., 1998); and alfalfa (Wang and Han, 2007)]. In addition to its effect on yield improvement and mineral composition, Spd also improved the salt-reduced red pigmentation of Pokkali rice pericarp. Pokkali grains have dark red pericarp which was much reduced in color intensity when the plants were subjected to NaCl and Spd (without NaCl). The red pigment proanthocyanidins, one of the three major classes of flavonoid pigments (flavonols, anthocyanins and proanthocyanidins), are oligomers or polymers of flavan-3-ol units synthesized via the flavonoid biosynthetic pathway

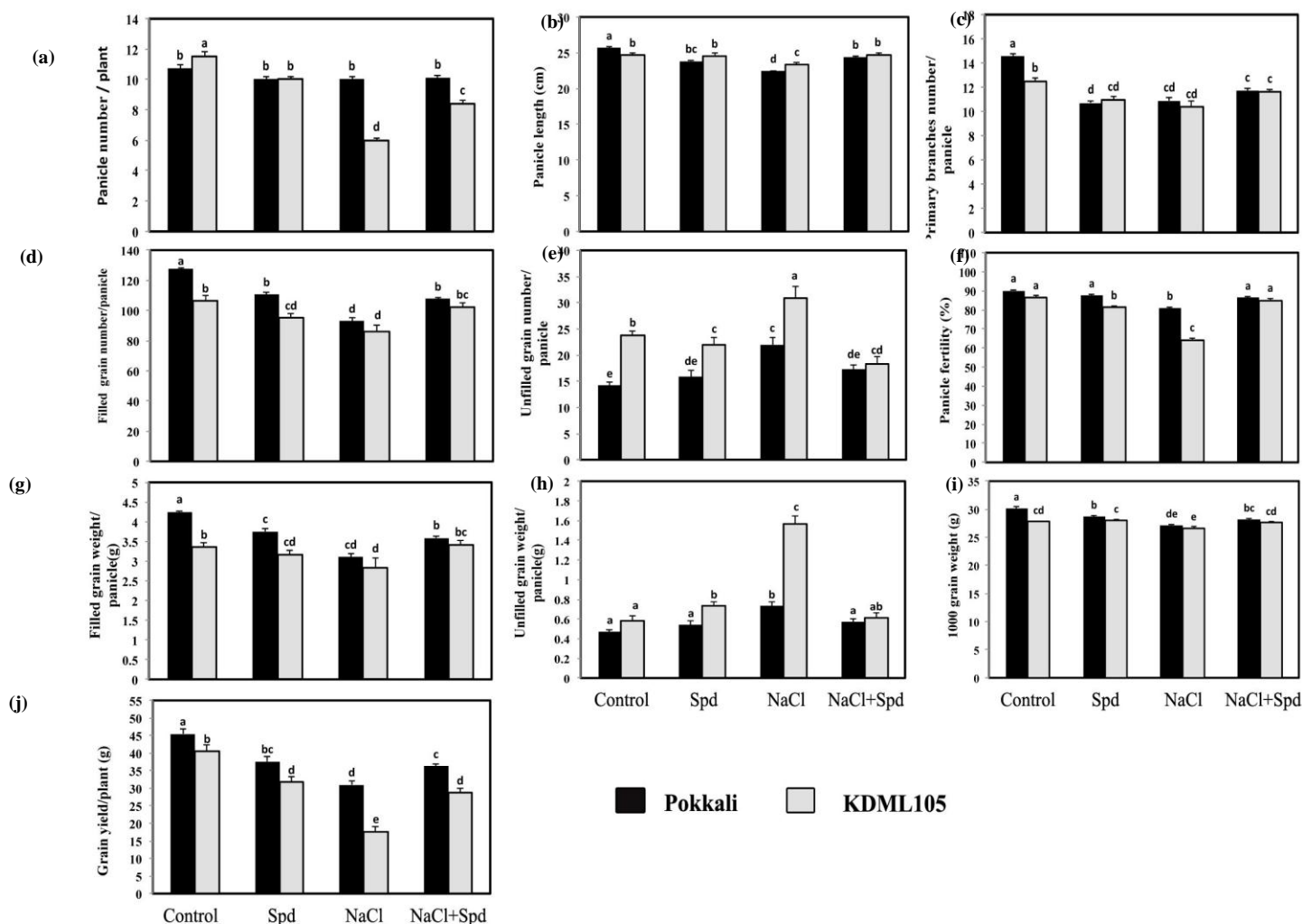


Fig 1. Effect of exogenous Spd (1 mM) on yield and yield components of rice cvs. Pokkali (black bars) and KDML105 (grey bars) exposed to 25 mM NaCl. (a), number of panicles per plant; (b), panicle length; (c), number of primary branches per panicle; (d), number of filled grains per panicle; (e), number of unfilled grains per panicle; (f), panicle fertility; (g), filled grain weight per panicle; (h), unfilled grain weight per panicle; (i), 1,000-grains weight; (j), grain yield per plant. The values shown are means \pm SE. Values with different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Control, plants were grown normally without Spd or NaCl until maturity; NaCl+Spd, plants were sprayed with 1 mM Spd for 7 days at the early booting stage and then were treated with 25 mM NaCl until maturity; NaCl, plants were treated with 25 mM NaCl without Spd pretreatment; Spd, plants were sprayed with 1 mM Spd for 7 days at the early booting stage and then watered normally.

(Dixon et al, 2005). All modern rice varieties with white pericarp, including KDML105 do not synthesize proanthocyanidin due to a mutation in the *Rc* gene encoding a positive regulator of proanthocyanidin synthesis (Sweeney et al, 2007). Biosynthesis of flavonoids including proanthocyanidins has been found to be influenced by numerous environmental factors including water stress (He et al, 2008). Ali et al (2007) found that salt stress had variable effects on flavonoid content of different organs of chamomile (*Chamomilla recutita*) and majoram (*Origanum majorana*) depending on the concentration of NaCl. In the cases where NaCl reduced the amount of flavonoids, exogenous PAs alleviated this effect. In other cases while low concentrations

of NaCl activated flavonoid accumulation, exogenous PAs resulted in a further increase in the amount of flavonoids, particularly in roots, shoots and flowers of *Origanum majorana*. Considerable attention has recently been drawn to proanthocyanidins and their monomers because of their potential beneficial effects on human health. Therefore, it is of great value to pursue further studies on the influence of environments and plant growth regulator such as PAs on biosynthesis of proanthocyanidins in plant fruits and seeds including rice grains with red pericarp. The aroma quality of fragrant rice depends on the concentration of 2-AP in mature grains which is controlled by both genetic and environmental factors.

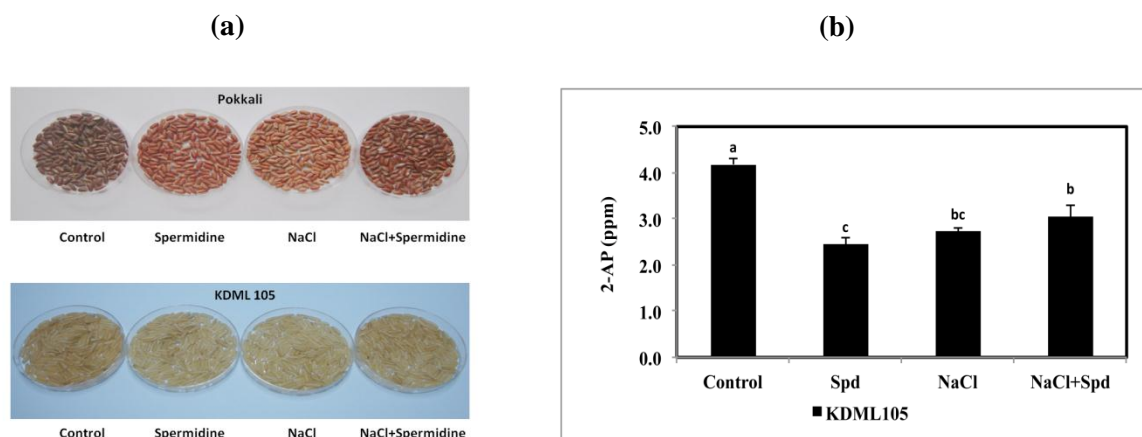


Fig 2. Effect of exogenous Spd pretreatment on (a), pericarp color of dehulled grains of Pokkali and KDML105 exposed to NaCl stress and (b), 2-AP content of dehulled grains of KDML105.

The genetic cause of fragrance in aromatic rice cultivars is due to the loss-of-function mutation in *badh2* gene leading to non-functional BADH2 enzyme or *Os2AP* gene blocking the conversion of proline to glutamic acid leading to an elevation of 2-AP (Bradbury et al., 2008; Chen et al., 2008; Vanavichit et al., 2008). Although the genetic factor plays a major role in determining the amount of 2-AP, environmental factors including water shortage and salinity-affected soils can influence the aroma quality of rice grains (Yoshihashi et al., 2004). Gay et al. (2010) found a positive correlation between 2-AP content and the electrical conductivity of the soil solution. However, these authors concluded that the increase of 2-AP with salinity was mainly due to a modification of some yield components and physical grain characteristics rather than the direct effect of salinity on 2-AP biosynthesis. Roychoudhury et al. (2008) reported that in the leaves of Gobindohog (aromatic rice), considerable aroma was detected after salinity stress, because elevated proline accumulation was induced after salt stress. Conversely, Fitzgerald et al. (2008) found no effect of salt stress on 2-AP content in leaf tissue of Jasmine and Basmati rice cultivars. In contrast to other studies we found that salt stress resulted in a significant reduction in 2-AP content in greenhouse-grown KDML105 rice plants. Therefore, more studies need to be done to improve our understanding of the effects of environmental conditions on the content 2-AP. The observation that Spd treatment increased the amount of 2-AP in salt-stressed plants is intriguing. Considering that Spd can be converted to putrescine, an intermediate of 2AP biosynthetic pathway and proline is accumulating under salt stress, it is interesting to perform a more detailed study to understand the interrelationship between 2-AP biosynthesis, polyamine catabolism and proline metabolism.

Materials and methods

Plant material, treatments, growth conditions and agronomic traits measurement

Two cultivars of rice (*Oryza sativa* L. ssp. *indica*), cv. Pokkali (salt-tolerant) and Khao Dawk Mali 105; KDML105 (salt-sensitive) were used in this study. The experiment was conducted in outdoor conditions in a wire house (with walls of nylon net) during the months of June to November 2010. The minimum and maximum temperature ranged from 21 to 24 °C and 32 to 36°C, respectively, and the mean relative humidity values were 78, 79, 81, 84, 76, and 70% in June, July, August, September, October and November,

respectively. The potting mix was composed of sandy clay loam soil and farm yard manure in the ratio of 3:1 (v/v). Ten kg of the potting mix was filled in each plastic pot (height: 0.30 m; diameter: 0.25 m). Five seeds were planted in each pot and 14 days after emergence the plants were thinned to three per pot. The water level in each pot was maintained at 5 cm above the soil surface using tap water. The plants were fertilized at the tillering stage with chemical fertilizer at the rate of 0.45 g per pot. At the panicle initiation stage, the plants were divided into four groups, one control and three treatments including (1) Spd, (2) NaCl and (3) NaCl + Spd. There were ten pots per treatment arranged in a CRD. For the Spd and the NaCl + Spd treatments, the plants were treated with 1 mM Spd by foliar spraying at 18.00 pm for 7 successive days. The control plants were sprayed with distilled water. The volume of Spd solution or distilled water used was 100 ml per pot. After the Spd treatment concluded, salinity treatment was imposed to the NaCl and NaCl + Spd treatments by adding 25 mM NaCl instead of tap water, while the control and the Spd treatments continued to be fed with tap water. At maturity, the panicles were harvested and threshed manually. Yield-related data including the number of panicles per plant, panicle length, number of primary branches per panicle, number of filled grains per panicle, number of unfilled grains per panicle, filled grains weight per panicle, unfilled grains weight per panicle, 1000-grain weight and grain weight per plant were recorded from 30 plants in each treatment.

Nutrient measurement

The grain husk was removed and the brown rice grains were ground with a mortar and pestle. For the analysis of Na, K, Ca, Mg, Fe, Mn, Cu and Zn using wet digestion method, five samples (0.5 g each) from five randomly selected plants of each treatment were placed in a digestion flask where 15 ml of HNO₃:H₂SO₄:HClO₄ (5:3:1) was then added and heated in a hot plate at 200°C for 2 hr until cleared. The solution was made up to 100 ml with deionised water (Hanlon, 1998; Horneck and Hanson, 1998). The amount of Na, K, Ca, Mg, Fe, Mn, Cu and Zn were determined using an atomic absorption spectrometer (Corning, Model GBC 932 AAA, England). Phosphorous was analyzed by vanado-molybdate method using a visible spectrophotometer. For determination of total N using a Kjeldahl method, five samples each of 0.5 g were digested with 10 ml H₂SO₄, catalysed by K₂SO₄:CuSO₄.5H₂O (10:1) and heated in a hot plate at 350°C for 12 hr. The solution was made up to 100 ml with deionised water and was taken up in 10 ml for distillation, and then the

solution was titrated with 0.005 N of H₂SO₄ for determination total N (Horneck and Miller, 1998).

2-acetyl-1-pyrroline (2-AP) content measurement

The grain husk of KDML105 rice was removed and the brown rice was ground with a mortar and pestle. Five samples (20 g per sample) of each treatment were analyzed for 2-AP by Head Space Gas Chromatography (HSGC) at the Science and Technology Service Center, Chiang Mai University (STSC-CMU), Thailand.

Statistical analysis of data

One way analysis of variance was performed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL). Significant difference between means among treatments were determined by Duncan's multiple range test (DMRT). The *p* value smaller than 0.05 was considered as statistically significant.

Conclusions

Salinity treatment imposed during the late booting stage through to maturity resulted in significant reductions in yield and yield-related parameters of both Pokkali and KDML105 rice cultivars, and pretreatment by foliar spraying of Spd to rice plants during the early booting stage significantly improved yields, particularly that of the salt-sensitive KDML105. Spd also increased K and reduced Na concentrations in the mature grains leading to higher K/Na ratio, but did not show beneficial effects on the improvement of other mineral nutrients. It is of great value to further investigate the effects of Spd, and other PAs, in relation to the concentrations and timing of PA application, levels and duration of salinity treatment and also on other aspects of grain quality. More detailed studies along this line may ultimately lead to an appropriate cultural practice leading to an improvement of rice yield in saline fields.

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