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Identification of Salt Tolerance in Thai Indigenous Rice on the Basis of the Na/K Ratio and Salt Stress Responses

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Abstract: Salinity responses to salinity stress in Thai indigenous rice and an improved variety (PTT1) were investigated. Pokkali (salt-tolerant) and IR29 (salt-sensitive) were used as standards. Sodium chloride at a concentration of 103 mM added into the Yoshida's nutrient solution was used for the salinity treatment and the responses were detected on day ten after salinity stress application. The classification of young rice seedlings for Na and K accumulation as well as physiological response based on salinity tolerances were then investigated. The results showed that all twelve varieties were classified into three main groups including tolerant, moderately tolerant and susceptible. Pokkali, PTT1 and the indigenous rice cultivars ULR198 and KKU-ULR076 were identified as salt tolerant. The salt-tolerant exhibited low Na content but accumulated high K resulting in a lower Na/K ratio, a higher survival rate and a lower salt injury score than the other varieties. These findings may be further employed for gene bank management on rice breeding programs.

Key words: Salt stress, salinity tolerance, indigenous rice, Na/K ratio

INTRODUCTION

Rice (*Oryza sativa*, L.) is one of the most important crops in Asia. Improved rice varieties have been mainly cultivated due to their grain milling quality compared to indigenous r ice lines. These situations may cause limitations in variations in the genetic diversity of rice. Studies have been focused on the characterization of native indigenous rice to obtain background data for breeding purposes (Venuprasad *et al*., 2008; Cairns *et al*., 2009; Li *et al*., 2011). Indigenous rice can be categorized into two main ecotypes which include upland rice and lowland rice. The indigenous upland rice is generally known as drought tolerant with some pathogenic tolerant aspects (Saito *et al*., 2010; Srihanoo and Sanitchon, 2011). It grows well in water deficit conditions, while lowland rice, especially the improved rice varieties, is grown in flooded paddy fileds (Wu *et al*., 2005).

Abiotic stresses such as drought, flooding, salinity and heat adversely affect both plant's vegetative and reproductive development. Saline toxicity occurred severely during water deficit periods as a result of accumulation of salt ions such as Na^+ and Cl^- on the soil surface (Cha-Um *et al.,* 2009; Kanjoo *et al*., 2011). Rice grown under salt stress also experiences some mineral deficiencies and toxicities (Gregorio *et al*., 2002). Reports proposed that the salt tolerant ability of rice is related to the vegetative growth of both shoot and roots and the ion balance between Na and K in shoot. The salt-tolerant lines take up higher concentrations of K in shoot than Na, resulting in adaptation under salt stress (Shaibur *et al*., 2008; Zhou *et al*., 2009). Characterization of indigenous rice lines is performed such as morphological traits, rice growth and development, crop productivity, germplasm screening and multiple stress tolerance identification for genetic improvement (Srihanoo and Sanitchon, 2011; Quijano-Guerta and Kirk, 2002; Gregorio *et al*., 2002).

The yield evaluation of 252 upland rice lines was investigated for rice breeding purposes by Srihanoo and Sanitchon (2011). It has been shown that upland rice produced a two times higher yield than the control lines. The authors suggested that indigenous rice lines might produce high yield and other unknown details should be further studied. Little is known about salt response in Thai indigenous upland lines of rice that have been identified into upland rice and PTT1 that differ in their salt tolerance under short-term salt stress. The present study was conducted to evaluate responses to salt stress of twelve rice cultivars which may show different levels in salt tolerance. Effects of salt stress were investigated at the young seedling stage on survival rate, salt injury score, total Na and K concentration in shoot of nine

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accessions of indigenous rice and PTT1 compared to salt-susceptible IR29 and salt-tolerant Pokkali under salt stress.

MATERIALS AND METHODS

Plant material: The plant materials used in this study include nine varieties of Thai indigenous rice (Table 1) kindly provided by Dr. Jirawat Sanitchon, Khon Khaen University, Thailand, an improved Thai variety (PTT1) and two standard varieties, the salt-sensitive IR29 and the salt-tolerant Pokkali, supplied by the Rice Gene Discovery Unit, BIOTEC, Kasetsart University, Thailand.

All rice varieties were grown in Yoshida's Nutrient solution (Gregorio *et al*., 1997) for two weeks by using Completely Randomized Design with three replications. NaCl at the concentration of 103 mM was added to the nutrient solution as salinity treatment. survival rate, Salt Injury Score (SIS), Na and K in shoot were determined after exposure to ten days of salt treatment. SIS was scored in numerals ranging from one to nine in accordance with the standard evaluation system of salt tolerance in rice seedlings. One represents the normal growth and tillering of rice while almost all dead and dying plants were scored nine (Gregorio *et al*., 1997; IRRI, 2002). Harvested plants were separated into root and shoot. The tissues were then oven-dried at 65°C for 48 h. The shoot Dry Weight (DW) was determined and delivered to Central Laboratory and Greenhouse Complex, Kasetsart University for Na and K quantification. Fifty miligram shoot dry weight was digested in 35% HNO_3 and resuspended in 10 mL 0.1 N HCl. The content of total Na and K in shoot (mg g^{-1}DW) was analyzed by atomic absorption spectrophotometry. Test of significance, correlation coefficients and cluster analysis were analyzed using SPSS version 20.

RESULTS

Screening for responses for salt tolerance of rice seedlings: All tested rice cultivars exhibited different visual symptoms after salinity stress. The salt injury score of young seedlings was observed to evaluate salt tolerance using Pokkali and IR29 as a standard check in salt screening. The results revealed that the most salinity tolerant types were Pokkali with a score of one and the second one was PTT1. Five moderately tolerant lines scored four to six were identified including ULR078, ULR192, ULR198, KKU-ULR076 and KKU-ULR198 (Fig. 1). In case of survival rate, four rice lines exhibited higher survival rates than the others. Their survival rates were calculated 72.8, 65.4, 62.1 and 58.2% for Pokkali, PTT1, ULR198 and KKU-ULR076, respectively (Fig. 1).

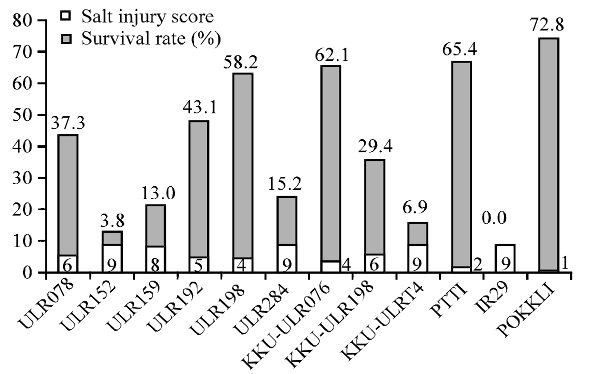

Fig. 1: Salt injury score and survival rate of all varieties at 10th day after salinization

Table 1: Rice used in this research

Designation	Local name	Source
ULR078	How Daeng	Surin, Thailand
ULR152	Upland rice	Loei, Thailand
ULR159	Upland rice	Loei, Thailand
ULR192	Upland rice	Phetchabun, Thailand
ULR198	Brown sticky rice	Phetchabun, Thailand
ULR284	Brown sticky rice	Phatthalung, Thailand
KKU-ULR076	How Klang	Phetchabun, Thailand
KKU-ULR198	Brown sticky rice	Phetchabun, Thailand
KKU-ULRT4	Chiang Mai	Chiang Mai, Thailand
PTT1	Pathumthani1	Pathumthani, Thailand
IR29	IR29	IRRI
Pokkali	Pokkali	IRRI

Na and K accumulation in shoot: The Na and K contents were detected on day ten after salt stress condition. The results showed that the non-stressed plants had taken up a higher concentration of K in shoot than Na but in the presence of excess Na, was absorbed at higher concentration. The salt-tolerant Pokkali contained 35.5 mg K g^{-1} DW and 14.4 mg Na g^{-1} DW while salt-sensitive IR29 contained 16.9 mg K g^{-1} DW and 23.5 mg Na g^{-1} DW in shoot tissues after treatment. The lowest Na/K ratio of 0.42 was found in Pokkali shoot under salt condition. Four rice varieties including PTT1, ULR198, ULR159 and ULR192 had lower Na/K ratios of 0.57, 0.67, 0.71 and 0.72, respectively compared to other tested cultivars (Fig. 2a-c). Results showed that K content revealed antagonistic relationships with Na content in shoot of rice seedling. The values of correlation coefficient (r) were calculated -0.56 and -0.55 for control and salt stress treatment, respectively (Fig. 3a, b). The data presented in Table 2 showed that the reduction of

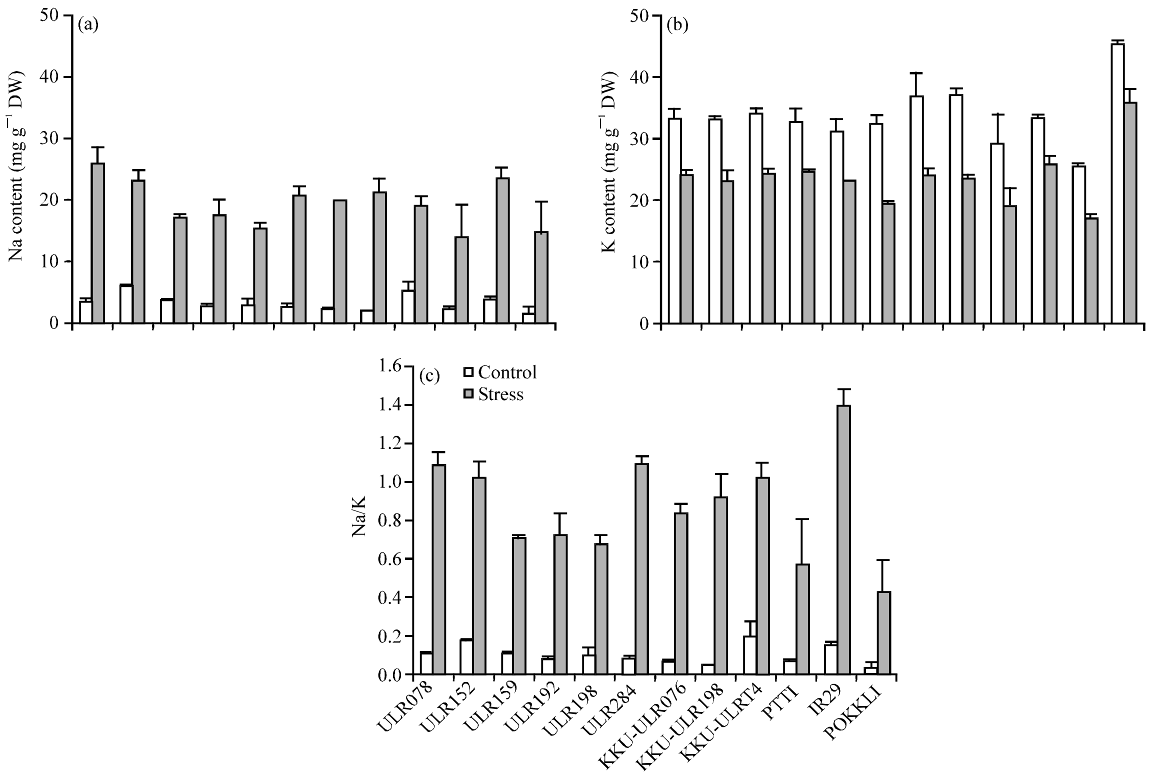

Fig. 2(a-c): Histogram illustrating the content of (a) Na, (b) K content (mg g^{-1} DW) and (c) Na/K ratio in shoot of young rice seedlings grown in nutrient solution containing 103 mM NaCl compared to the control

Table 2: Correlations between the content of Na and K, Na/K ratio, salt injury score and survival rate after ten days of salt treatment

Treatment	Na ---mg g^{-1} DW---	K	Na/K	Salt injury score	Survival (%)
Na (mg g^{-1} DW)	1	-0.564	0.877**	0.660*	-0.622*
K (mg g^{-1} DW)		1	-0.829**	-0.799**	0.730**
Na/K			1	0.813**	-0.781**
Salt injury score				1	-0.975**
Survival (%)					1

**Significant at the 0.01 level, *Significant at the 0.05 level

Na/K ratio was significantly correlated ($p<0.01$) to a lower salt injury score and a higher survival rate of rice under salt stress condition.

Clustering analysis of thai indigenous rice: Nine indigenous varieties, PTT1, Pokkali and IR29 were analyzed for Na and K accumulation as well as physiological responses based on salinity tolerances at the young seedling stage. The results showed that all varieties were classified into three main groups including tolerant, moderately tolerant and susceptible (Fig. 4). It was found that out of nine indigenous varieties, two were tolerant, three were moderately tolerant and four were susceptible. Pokkali, PTT1, ULR198 and KKU-ULR076 were classified in the salt tolerant group while IR29, ULR152, ULR159, ULR284 and KKU-ULRT4 were in the susceptible group. The salt tolerant rice exhibited some important characteristics such as a lower Na/K ratio in shoot, a higher survival rate and a lower salt injury score than the sensitive plants. In addition, ULR078, ULR192 and KKU-ULR198 were identified as moderately tolerant cultivars in this study.

DISCUSSION

All twelve rice were classified into three main groups including tolerant, moderately tolerant and susceptible. The tolerant rice exhibited a lower Na/K ratio in shoot, a higher survival rate and a lower salt injury score. Salt toxicity severely affected sensitive rice seedlings and could reduce the number of surviving plants. K accumulation in shoot was mainly more activated than Na for the salt tolerant lines including Pokkali, PTT1, ULR198 and KKU-ULR076. Typically, K^+ is a major element and is required for many metabolic reactions in plants, but Na^+ is not an essential element for most plants. The higher salt tolerance of plants is positively related to maintaining

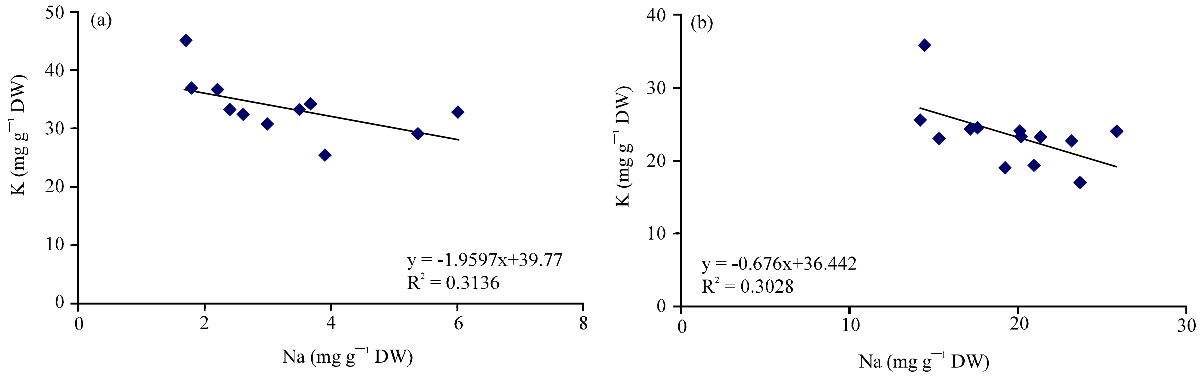

Fig. 3(a-b): Relationships between Na and K content (mg g^{-1}DW) in shoot of, (a) Control and (b) Salt stress treatment

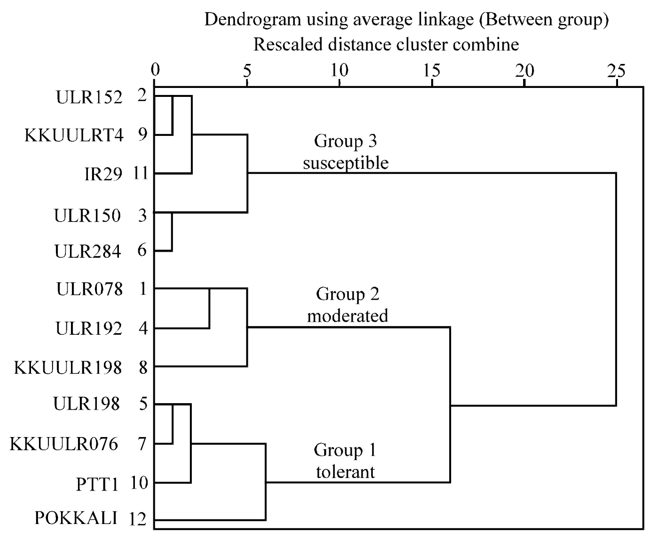

Fig. 4: Dendogram showing physiological similarities between examined lines based on Na and K accumulation and physiological responses using the SPSS program (version 20)

Na^+/K^+ homeostasis under salt stress (Zhou *et al*., 2009; Summart *et al*., 2010; Maggio *et al*., 2002). Reports showed that K^+ reveals antagonistic relationships with Ca, Mg and Na in rice seedlings regulating for the reduction of ion toxicity in plant tissues (Shaibur *et al*., 2008; Aleman *et al*., 2009). Results suggest that seedlings of Pokkali, PTT1, ULR198 and KKU-ULR076 could develop the tolerance ability to 103 mM NaCl under this experimental condition. Moreover, Thai indigenous rice, ULR198 and KKU-ULR076 and an improved variety PTT1 are considered potentially tolerant to salt stress at the seedling stage, comparable to Pokkali. These findings may be further employed for gene bank management in rice breeding programs.

CONCLUSION

Two indigenous rice including ULR198 and KKU-ULR076 and an improved variety PTT1 were identified as salt tolerant under the salt stress compared to Pokkali. These rice exhibited low Na content but accumulated high K resulting in a lower Na/K ratio, a higher survival rate and a lower salt injury score than the other varieties.

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