The Effect of Salt Stress on Productivity, Grain Morphology, Grain Carbohydrate and Storage Protein Accumulations of 4 Rice (*Oryza sativa* L.) Cultivars Differing in Degrees of Salt Tolerance

ผลของความเครียดเกลือต่อความสามารถในการให้ผลผลิต ลักษณะสัณฐานของเมล็ด ปริมาณสารคาร์โบไฮเดรตและโปรตีนสะสมในข้าว (*Oryza sativa* L.) 4 พันธุ์ ที่มีความสามารถในการทนเค็มแตกต่างกัน

Wichian Sangwongchai (วิเชียร แสงวงษ์ชัย)* Dr.Maysaya Thitisaksakul (ดร.เมศยา ธิติศักดิ์สกุล)**,***

ABSTRACT

The purpose of this research is to examine the effect of moderate salt stress (EC = 4.0 dSm^{-1}) on productivity, grain morphology, and some changes in biochemical composition of 4 rice cultivars having different degrees of salt tolerance, including Pokkali (salt-tolerant), RD15 (moderately salt-tolerant), KDML105 (moderately salt-susceptible) and IR29 (salt-susceptible). Salt stress was initiated at the anthesis stage and was maintained until harvest. The results showed that the salt stress at anthesis stage decreased panicle numbers per plant, total grain weight, 100-grain weight, grain fertility, and grain volume of RD15 and KDML105 cultivars ($p < 0.05$). However, the measurement of storage biomolecule content showed that the grain reducing sugar content was not significantly different between the treatment and control group in all cultivars. Interestingly, salt stress enhanced the starch content in the grain of RD15 and KDML105 ($p < 0.05$), while the total storage protein content was increased in the grain of IR29 ($p < 0.05$), which was a consequence of an increase in the amount of glutelin large subunit.

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของความเครียดเกลือในระดับปานกลาง (EC = 4.0 dSm^{-1}) ต่อความสามารถในการให้ผลผลิต ลักษณะสัณฐานของเมล็ด และการเปลี่ยนแปลงองค์ประกอบของสารชีวโมเลกุลบางประการในข้าว 4 พันธุ์ที่มีความสามารถในการทนเค็มแตกต่างกัน ได้แก่ พันธุ์ Pokkali (ข้าวพันธุ์ทนเค็ม) พันธุ์ RD15 (ข้าวพันธุ์ทนเค็มปานกลาง) พันธุ์ KDML105 (ข้าวพันธุ์ที่อ่อนไหวต่อความเค็มปานกลาง) และพันธุ์ IR29 (ข้าวพันธุ์ที่อ่อนไหวต่อความเค็ม) โดยเริ่มให้ความเครียดเกลือแก่ต้นข้าวตั้งแต่ระยะสืบพันธุ์และรักษาระดับความเครียดเกลือจนถึงระยะเก็บเกี่ยว ผลการทดลองพบว่าความเครียดเกลือในระยะสืบพันธุ์ส่งผลให้จำนวนรวง น้ำหนักเมล็ดทั้งหมด น้ำหนัก 100 เมล็ด ความเจริญพันธุ์ของเมล็ด และปริมาตรของเมล็ด ของข้าวพันธุ์ RD15 และ KDML105 ลดลง ($p < 0.05$) และจากการตรวจวัดปริมาณสารชีวโมเลกุลสะสมพบว่าปริมาณน้ำตาลรีดิวซ์ในเมล็ดข้าวทั้ง 4 พันธุ์จากทั้งสองกลุ่มทดลองไม่แตกต่างกัน แต่กลับพบว่าความเครียดเกลือส่งผลให้ปริมาณสตาร์ชในเมล็ดของข้าวพันธุ์ RD15 และ KDML105 เพิ่มสูงขึ้น ($p < 0.05$) ในขณะที่ปริมาณโปรตีนสะสมในเมล็ดของข้าวพันธุ์ IR29 เพิ่มสูงขึ้น ($p < 0.05$) ซึ่งเป็นผลจากการเพิ่มขึ้นของปริมาณซับยูนิทขนาดใหญ่ของโปรตีน glutelin

Keywords: rice, saline soil, salt stress

คำสำคัญ: ข้าว ดินเค็ม สภาวะเครียดเกลือ

** Student, Master of Science Program in Biochemistry, Faculty of Science, Khon Kaen University*

*** Doctor, Department of Biochemistry, Faculty of Science, Khon Kaen University*

**** Salt-tolerant Rice Research Group, Khon Kaen University*

Introduction

Rice (*Oryza sativa* L.) is the main staple for people that live around the world and is one of the highest food demands of Asian people's lives. Each year, demands of rice are at least 617 million tons worldwide. Rice is grown in almost all continents with approximately 958 million acres of plantation land, by which about 90 percent are produced and consumed in Asia (Ministry of agriculture and cooperatives, 2007). However, the production of rice in several areas is threatened by the salinization of the lands (IRRI, 2006). Climate change and the continuous increase of the sea level due to a combination of the greenhouse effect and inappropriate agricultural practices have led to the increase in soil salinity, especially in the coastal areas (Thitisaksakul *et al*., 2015). Soil salinity with the electrical conductivity of about 2 dSm^{-1} could create a loss of rice yield up to a ton per hectare (Asch and Wopereis, 2001). The increase of saline water in the soil directly results in salt stress in plants. When the large amounts of ions are present in the soil, the two problems arise including: (1) the high concentration of salt decreases the water potential in the rhizosphere, which prevents water absorption by the root, and (2) the accumulation of Na^{+} and Cl^{-} ions, which is toxic to plant cell (Parida and Das, 2005). Thereby, salt stress is a threat to crop production and is one of the major causes of agricultural loss worldwide (Flowers and Flowers, 2005).

Saline soil is one of the most important factors limiting productivity of rice. It causes grain width-to-length ratio and volume decrease (Thitisaksakul *et al*., 2015) as well as other symptoms such as low germination rate, shortened height, low tillering, spikelet sterility, low grain weight, and limited root growth (IRRI, 2006). Moreover, besides lowering the rice yield, salinity also affects carbohydrate and protein accumulation in the grain (Thitisaksakul *et al*., 2015). The changes in carbohydrate content however depend on the rice cultivars and stress conditions. Siscar-Lee *et al*. (1990) found a general decrease in starch accumulation in rice grains due to salt stress. On the other hand, it has been reported that starch contents in the grains of Nipponbare increase under low salt stress (Thitisaksakul *et al*., 2015). Salinity stress at the electrical conductivity (EC) of 4-8 dSm^{-1} increased protein accumulation in rice grain (Rao *et al*., 2013; Baxter *et al*., 2011). The changes of these biomolecules composition in the grain affect test and rice sensory quality (Siscar-Lee *et al*., 1990; Rao *et al*., 2013). Therefore, the fight against saline soil is urgently needed to reduce the risk of agricultural losses (Hariadi *et al.,* 2015). The use of rice salt tolerant cultivars in a breeding program to introduce salt-tolerant phenotypes into the commercial cultivars is one of the most promising methods (Zhikang and Jianlong 2011). A previous research reporting on the evaluation of salt tolerance in 30 rice cultivars at seedling stage had classified them into 5 groups according to the degrees of salt tolerance including salt tolerant (T), medium salt tolerant (MT), medium susceptible (MS), susceptibility (S) and high susceptibility (HS) (Kanawapee *et al*., 2011). The information on how these different groups respond to the salt stress in terms of productivity and grain quality will be vital for the salt-tolerant breeding programs.

In the present research, we aimed to understand the effect of salt stress on productivity, grain morphology, and grain carbohydrate and protein accumulations in 4 rice cultivars differing in 4 levels of salt tolerance. We also evaluated the relationship between the degree of salt tolerance, and the ability of plant to maintain growth, yield and quality of grain.

Objective of the study

The aim of this research was to determine the effect of salt stress on productivity, grain morphology, and grain carbohydrate and protein accumulations of 4 rice cultivars differing in 4 levels of salt tolerance, and to understand the relationship between degree of salt tolerance, and changes in yield, grain morphology, and grain storage biomolecules content under salt stress.

Materials and methods

Plant materials and growth conditions

Rice seeds of 4 cultivars, which are Pokkali, RD15, KDML105 and IR29, were germinated in pretri dishes. They were then transplanted into 72 8□ plastic pots and placed in a greenhouse under natural light conditions at the Faculty of Science, Khon Kaen University from February to June 2018. The pots of the rice plants were divided into 2 groups. At anthesis, the salt treatment group was treated with 40 mM NaCl (EC = 4 dSm^{-1}) until harvest. For the control group, the EC value of water was maintained at 0 dSm^{-1} until harvest. For productivity and grain quality, nine replicates per treatments x genotypes were studied, and 4 replicates were used for the biochemical analyses. At harvest time, the water was removed from the pots, and the panicles were counted and harvested after the rice plants were left in the pots for 7 days (Thitisaksakul *et al*., 2015).

Study of rice productivity and grain morphology

The number of panicles, grain weight, and grain fertility were assessed to calculate the average number of panicles per plant, total grain weight, 100-grain weight, and seed fertility (expressed as number of fully filled seeds per 100 seeds) (Thitisaksakul *et al*., 2015). Grain dimensions of 10 seeds per plant were measured using Vernier calipers. The grain width, length, width-to-length ratio, perimeter and volume were recorded (Thitisaksakul *et al*., 2015).

Extraction and measurement of grain reducing sugar and starch content

Reducing sugar was extracted from 200 mg rice flour by boiling in 2 mL 80% (v/v) ethanol 3 times. Then, 300 μL of the supernatant containing grain sugars was dried using Speed Vac (LaboGene, India). It was resuspended with 100 μL distilled water and kept at 4 °C for sugar assay. The ethanol insoluble pellet was finely ground using an all-glass homogenizer and was then digested using an enzyme cocktail (2U α-amylase and 12U amyloglucosidase) at 37 °C for 24 h (Luengwilai and Beckles, 2010). The quantity of reducing sugar in both fractions was analyzed by 3,5-dinitrosalicylic acid (DNS) method with glucose as standards (Miller, 1959).

Measurement of grain storage protein content by BCA (Bicinchoninic Acid) protein assay and SDS-PAGE

Grain protein was extracted following the method of Shyur et al. (1988). Approximately 40 mg of rice grain was incubated in an extraction buffer (4 M urea, 2% SDS, 62.5 mM Tris HCl pH 6.8, 10% glycerol and 5% β-mercaptoethanol) at -20 °C for 24 h. The protein content was then analyzed using the BCA assay with bovine serum albumin (BSA) as the standard protein. Subsequently, discontinuous SDS-PAGE using 4% stacking and 15% separating gels was performed at 180 V, following the method of Leammli (1970). The gel image was captured using the Gel Doc (G: BOX F^3- Syngene, US). The protein band intensity was measured using the ImageJ v.1.52 software (NIH, US) (Rasband, 2018).

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to determine statistically significant differences between means of treatments x cultivars (p < 0.05). A Tukey-Kramer post hoc test is calculated when statistical differences were identified. For both analyses, the SPSS® statistics v.23 software was used (IBM, US).

Results

The effect of salt stress on yields and grain characteristics

The rice grains at harvest were displayed in Figure 1 and the effect of salt stress on panicle number per plant, total grain weight, 100-grain weight and the percentage of grain fertility was shown in Table 1. The panicle number per plant, total grain weight, 100-grain weight and the percentage of fertility of RD15 and KDML105 cultivars in the salt treatment group were lower than those of the control group. However, the panicle numbers per plant, total grain weight, 100-grain weight and the percentage of fertility of Pokkali and IR29 cultivars were not affected by salt treatment (Table 1). Many infertile unfilled grains of RD15 and KDML105 cultivars - shown by white hull color, in the salt treatment group obviously increased when compared to the same cultivars in control group (Figure 1). Table 2 displays the effect of salinity stress on grain characteristics. The grain width of Pokkali and RD15 cultivars was declined by moderate salt treatment, while that of KDML105 and IR29 cultivars remained unaffected (Figure 1; Table 2). In addition, the grain length, perimeter and grain volume of RD15 and KDML105 cultivars in the salt treatment group decreased when compared to those of the control group (Table 2), while the grain length of Pokkali and IR29 cultivars was not affected by salt treatment (Table 2). The width-to-length ratio of Pokkali cultivar in the salt treatment group decreased when compared to that of the control group. However, the width-to-length ratio of RD15, KDML105 and IR29 was not affected by moderate salt stress (Table 2).

Table 1 Productivity of four rice cultivars cultivated under control and saline conditions

Treatments	Cultivars	Panicle number	Total grain weight (g)	100-grain weight (g)	%Fertility
Control	Pokkali	7.33 ± 0.50^{b}	11.42 ± 0.83^{a}	2.07 ± 0.08^{a}	65.22 ± 3.57^{a}
	RD15	4.75 ± 0.72^{cd}	4.32 ± 0.75^{de}	1.17 ± 0.05^{c}	31.17 ± 3.63^{b}
	KDML105	6.00 ± 0.37^{bc}	6.42 ± 0.51^{cd}	1.29 ± 0.09^{c}	35.33 ± 4.31^{b}
	IR29	9.85 ± 0.40^{a}	8.49 ± 0.50^{bc}	1.51 ± 0.05^{bc}	71.28 ± 3.39^{a}
Salt	Pokkali	7.44 ± 0.33^{b}	10.41 ± 0.47^{ab}	1.84 ± 0.09^{ab}	57.33 ± 3.33^{a}
	RD15	1.83 ± 0.65^{e}	0.75 ± 0.18^{f}	0.55 ± 0.04^{d}	4.00 ± 2.08^{c}
	KDML105	2.77 ± 0.66^{de}	1.77 ± 0.52^{ef}	0.68 ± 0.09^{d}	10.00 ± 4.29^{c}
	IR29	9.88 ± 0.38^{a}	8.99 ± 0.58^{ab}	1.50 ± 0.05^{bc}	75.44 ± 3.33^{a}

Values are means ± SEM. Means within each column with different superscripts are significantly different (p < 0.05) by Tukey's test (n = 9).

Figure 1 Effect of salt stress at anthesis stage on grain characteristic of four rice cultivars. Thirty random grains of each treatment were shown.

Table 2 Grain characteristics of four rice cultivars cultivated under control and saline conditions

Treatments	Cultivars	Width (mm)	Length (mm)	Width-to-length ratio	Perimeter (mm)	Volume (mm^3)
Control	Pokkali	2.91±0.010^{a}	5.57±0.025^{e}	0.52±0.002^{a}	13.32±0.050^{de}	24.77±0.251^{a}
	RD15	2.03±0.005^{c}	6.96±0.040^{ab}	0.29±0.001^{d}	14.13±0.065^{ab}	15.06±0.128^{bc}
	KDML105	2.04±0.006^{c}	7.06±0.034^{a}	0.29±0.001^{d}	14.30±0.058^{a}	15.43±0.138^{b}
	IR29	1.98±0.010^{d}	5.98±0.041^{d}	0.33±0.002^{c}	12.50±0.070^{f}	12.29±0.165^{e}
Salt	Pokkali	2.85±0.014^{b}	5.59±0.030^{e}	0.51±0.003^{b}	13.27±0.058^{e}	23.96±0.303^{a}
	RD15	1.97±0.013^{d}	6.70±0.058^{c}	0.30±0.002^{d}	13.62±0.101^{cd}	13.69±0.238^{d}
	KDML105	2.01±0.011^{cd}	6.82±0.055^{bc}	0.29±0.002^{d}	13.86±0.097^{bc}	14.42±0.238^{cd}
	IR29	1.98±0.008^{d}	6.05±0.034^{d}	0.33±0.002^{c}	12.62±0.058^{f}	12.45±0.130^{e}

Values are means ± SEM. Means within each column with different superscripts are significantly different ($p < 0.05$) by Tukey's test (n = 9). Ten brown rice grains were randomly studied for each biological replicate.

The effect of salt stress on grain carbohydrate and storage protein contents

Rice starch is important for its used as food materials (Brady and Yoshida, 1979). The analysis of the amount of starch and reducing sugar in the grains of four rice cultivars was conducted, since the mechanism of carbohydrate synthesis and accumulation in endosperms might be affected by salt stress (IRRI, 2006). We did not find significant difference in the grain reducing sugar content in all cultivars (Table 3). Interestingly, the starch content in the grain of RD15 and KDML105 cultivars in the salt treatment group significantly increased when compared to the control group, while the starch content in the grain of Pokkali, and IR29 cultivars was not changed by salt stress (Table 3).

Grain storage protein is another component that could affect its end-uses and cooking quality (IRRI, 2006). Protein synthesis mechanism might also be affected by salt stress (Kumar *et al.,* 2017). Thus, the effect of salt stress on storage protein alteration was investigated. It was found that the total storage protein in the grain of IR29 was increased by the salt stress treatment (Table 3). This is consistent with results from SDS-PAGE analysis, which showed that the glutelin large subunit (LS) of salt-treated IR29 grains was enhanced by moderate salt stress (Figures 2A and 2B). However, the protein band intensity of the glutelin small subunit (SS) and prolamin from the grains of IR29 cultivar was not altered by salt stress (Figures 2C and 2D). In addition, the total storage protein and the protein band intensity of all storage proteins in the grain of Pokkali and KDML105 cultivars were not affected by moderate salt stress.

Table 3 Storage biomolecules composition in the grain of four rice cultivars cultivated under different salinity conditions

Storage biomolecules composition	Treatments	Cultivars			
		Pokkali	RD15	KDML105	IR29
Reducing sugar content ($mg\ gFW^{-1}$)	Control	1.60±0.11	0.94±0.07	0.88±0.07	0.97±0.09
	Salt	1.64±0.15	0.94±0.06	0.80±0.09	1.00±0.07
Starch content ($mg\ gFW^{-1}$)	Control	632.34±20.06^{b}	614.64±17.95^{bc}	510.08±11.10^{d}	559.85±5.69^{bc}
	Salt	625.93±10.45^{bc}	853.15±51.16^{a}	539.79±2.46^{bc}	532.53±3.66^{cd}
Protein content ($mg\ gFW^{-1}$)	Control	56.69±1.58^{a}	-	27.26±1.85^{c}	30.04±2.33^{c}
	Salt	56.73±1.60^{a}	-	31.83±1.25^{c}	42.98±0.89^{b}

Values are means ± SEM. Means within each column with different superscripts are significantly different ($p < 0.05$) by Tukey's test (n = 4 for starch content analyses; n = 3 for protein content analysis). The protein content analysis of RD15 cultivar was not carried out due to the limited amount of harvested grains.

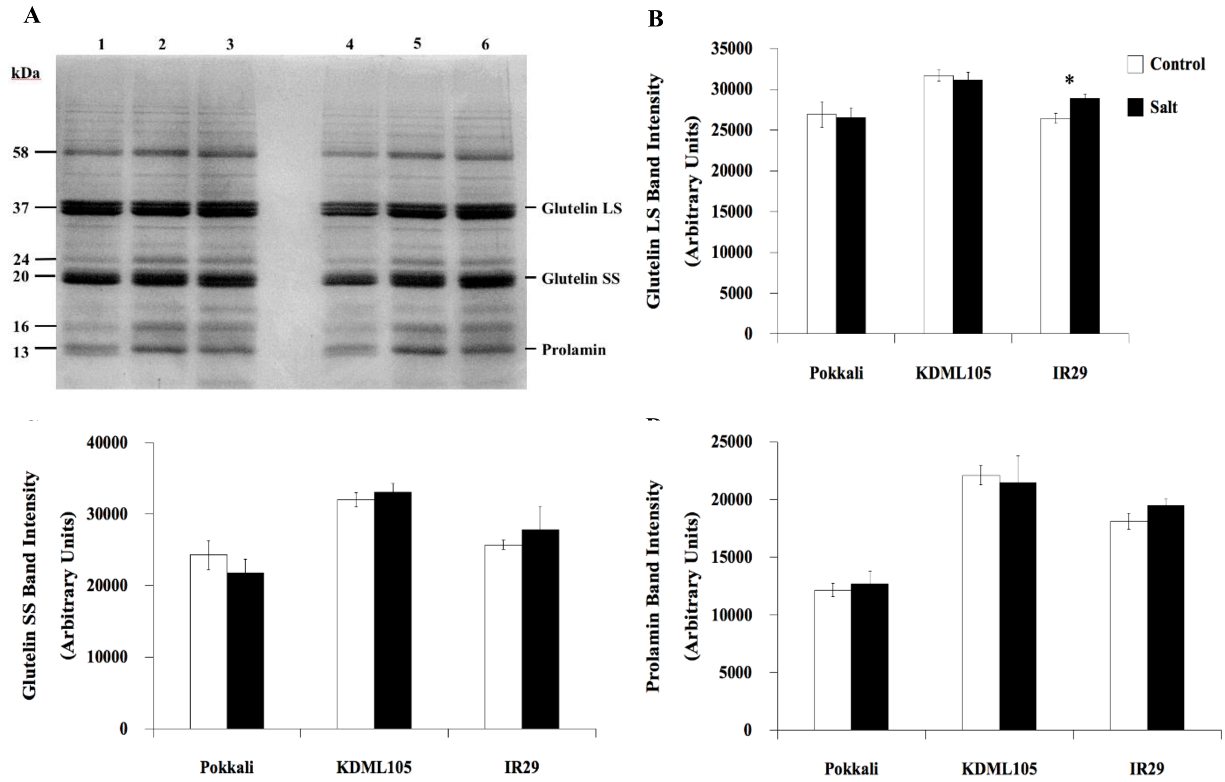

Figure 2 Analysis of grain storage proteins extracted from the flour of three rice cultivars cultivated under control and saline conditions. A: The SDS-PAGE analyses of grain storage proteins from cultivars Pokkali (lane 1), KDML105 (lane 2) and IR29 (lane 3) in the control group, and from Pokkali (lane 4), KDML105 (lane 5) and IR29 (lane 6) in the salt treatment group. The protein bands of the glutelin large subunit (LS), glutelin small subunit (SS), and prolamin were shown. B, C, D: Densitometry plot showing the relative content of glutelin large subunit (LS), glutelin small subunit (SS), and prolamin proteins, respectively. Values are means ±SEM. Means of each pair of columns with a star are significantly different ($P < 0.05$) by Student's t-test (n = 3).

Discussion

The anthesis stage is one of the most salt susceptible stages for rice plant (Hussain *et al*., 2017). In addition, rice genetic resources provide a wide collection of cultivars with different degrees of salt tolerance (Kanawapee *et al*., 2011). Understanding how these different rice cultivars respond to the salt stress initiated at its most sensitive stage is therefore necessary for any attempt to improve salt-tolerant rice cultivars. In this work, we determined the effect of salt stress on productivity, grain morphology, and grain carbohydrate and protein accumulations of four rice cultivars differing in four levels of salt tolerance. The result showed that salt stress at anthesis stage caused significant decrease in the panicle numbers per plant, total grain weight, and 100-grain weight and the percentage of fertility of RD15 and KDML105 cultivars (Table 1). Also, grain completeness of these two cultivars clearly reduced as shown by the significant reduction in the grain length, perimeter and grain volume (Table 2). On the other hand, Pokkali and IR29 cultivars maintained their productivity and grain morphology under moderate salt stress. This result is of great interest since IR29 is a standard salt susceptible cultivar (Kanawapee *et al*., 2011), but it maintained productivity and grain characteristics under the moderate salt stress in this study. The productivity of IR29 was also higher than RD15 and KDML105, both of which are photoperiod sensitive rice cultivars (Taprab *et al*., 2011). The long-day period (February to June) might delay the flowering time of RD15 and KDML105 (Swaminathan, 1986), and led to its lower productivity compared to IR29 and Pokkali, which are photoperiod insensitive cultivars (Khush and Virk, 2005; Waziri *et al*., 2016). Interestingly, moderate salt stress (EC 4) significantly increased the starch content in the grain of RD15 and KDML105 cultivars, while the reducing sugar content in the grain of RD15 and KDML105 cultivars was not affected by such saline condition (Table 3). This result is consistent with the findings from previous study, which reported that mild salt stress (EC 2) induced increased percentage of starch in the Nipponbare rice flour (Thitisaksakul *et al.,* 2015). High level of salt stress increased activity of ADP-glucose pyrophosphorylase (AGPase) in tomato (*Solanum lycopersicum*) fruits during the initial developmental stages, which enhanced starch accumulation in fruits (Yin *et al*., 2010). This could also be true for the results seen in RD15 and KDML105. Apart from the alteration in carbohydrate content, cultivating rice under salt stress conditions can increase total protein content in the grain, which generally resulted from the increase in the amount of glutelin protein (Baxter *et al*., 2011). In our present study, the enhanced protein accumulation was prominent in the grains of IR29 cultivar, which was consistent with the increase of the glutelin large subunit (LS) (Figures 2A and 2B). The increase in storage protein content could affect the cooking quality of rice, increasing its hardness upon cooking (Thitisaksakul *et al*., 2015), although other factors such as amylose content, starch granule size and distribution, and amylopectin side chain length distribution must be considered to draw such conclusion.

Conclusion

This study aimed to understand how different rice cultivars with different degrees of salt tolerance respond to moderate salt stress at anthesis stage. Overall, the moderate salt stress at anthesis stage did not have an influence on productivity, grain characteristics, and grain carbohydrate and protein accumulation of Pokkali cultivar, which is a salt-tolerant rice cultivar. Interestingly, the productivity, grain characteristics and grain carbohydrate accumulations of IR29 (salt-susceptible cultivar) were not affected by the salt stress implemented in this study. On the other hand, salt stress affected storage protein accumulations in the grain of IR29 cultivar, which led to the increase of the total protein content in the grain of IR29, suggesting that its protein biosynthesis might be more susceptible to salinity than the carbohydrate counterparts. Moreover, salt stress at anthesis stage greatly compromised the productivity and grain characteristics of RD15 and KDML105 cultivars. However, whether the decrease in yield was in part due to their sensitivity to photoperiod has to be determined in further study. Interestingly, starch accumulation in the grains of RD15 and KDML105 cultivars were enhanced by salt stress, suggesting a morphological adaptation of these two cultivars to moderate salt stress at reproductive stage.

Acknowledgement

This work is supported by the Research and Technology Transfer Affairs, Khon Kaen University. We thank Assoc. Prof. Dr. Piyada Theerakulpisut for a gift of rice seeds used in this study. We also thank Asst. Prof. Dr. Paweena Pongdontri for a gift of chemical reagents used in the protein assays. Lastly, we thank Mr. Wichit Taron for his technical assistance on SDS-PAGE analysis.

References

Asch F, Wopereis MCS. (2001). Responses of feld-grown irrigated rice cultivars to varying levels of floodwater salinity in a semi-arid environment. Field Crops Research 2001; 70(10): 127-137.

Baxter G, Zhao J, Blanchard C. Salinity alters the protein composition of rice endosperm and the physicochemical properties of rice flour. J. Sci. Food Agric 2011; 1 91: 2292-2297.

Brady NC, Shouichi Y. Fundamentals of rice crop science. The International Rice Research Institute 1979; 1-279.

Flowers TJ, Flowers SA. Why does salinity pose such a difficult problem for plant breeders?. Agricultural Water Management 2005; 78: 15-24.

Hariadi YC, Nurhayati AY, Soeparjono S, Arif I. Screening six varieties of rice (Oryza sativa) for salinity tolerance. Procedia Environmental Sciences 2015; 28(9): 78-87.

Hussain, S., Jun-hua, Z., Chu. Z., Lian-feng, Z., Xiao-chuang, C., Sheng-miao, Y., James, A. B., Ji-jie, H., Qian-yu, J. (2017). Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. Journal of Integrative Agriculture, vol.16(11). pp. 2357–2374.

IRRI (International Rice Research Institute). (2006). Breeding for grain quality. http://www. knowledgebank.irri.org/ricebreeding course/Grain_quality.htm. [Accessed: 28-September-2018].

IRRI (International Rice Research Institute). (2006). Stress and disease tolerance. http://www. knowledgebank.irri.org/ricebreedingcourse/bodydefault.htm#Breeding_for_salt_tolerance.htm. [Accessed: 18-Deceember-2018].

Kanawapee N, Sanitchon J, Srihaban P, Theerakulpisut P. Genetic diversity analysis of rice cultivars (Oryza sativa L.) differing in salinity tolerance based on RAPD and SSR marker. Electronic Journal of Biotechnology 2011; 14: 6.

Khush GS, Virk PS. IR varieties and their impact. Los Baños, Philippines: International Rice Research Institute; 2005. pp. 163

Kumar Y, Singh A, Matta NK. Proteomics of barley grains under varying salinity levels. Journal of Proteins and Proteomics 2017; 8(1): 49-63.

Leammli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685.

Luengwilai K, Beckles DM. (). Climacteric ethylene is not essential for initiating chilling injury in tomato (Solanum lycopersicum) cv. Ailsa Caig. Journal of Stored Products and Postharvest Research. 2010; 1(1): 1-8.

Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959; 31: 426-428.

Ministry of agriculture and cooperatives.). Thai rice strategy 2007-2011. Bangkok: Rice department; 2007. p. 19.

Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 2005; 60: 324-349.

Rao PS, Mishra B, Gupta SR. Effects of Soil Salinity and Alkalinity on Grain Quality of Tolerant,Semi-Tolerant and Sensitive Rice Genotypes. Rice Science 2013; 20(4): 284-291.

Rasband W. ImageJ 1.52. National Institutes of Health, US. [Online] 2018 [cited 2018 August 20]. Available from: http://imagej.nih.gov/ij/

Shyur LF, Zia KK, Chen CS. (1988). Purification and some properties of storage proteins in japonica rice. Bot.Bull.Academia Sinica 1988; 29: 113-122.

Siscar-Lee JJH, Juliano BO, Qureshi RH, Akbar M. Effect of saline soil on grain quality of rices differing in salinity tolerance. Plant Foods Hum Nutr 1990; 40: 31-36.

Swaminathan MS. The Flowering Response of the Rice Plant to Photoperiod. International Rice Research Institute; 1986: p. 1-66.

Taprab S, Sukviwat W, Chettanachit D, Wongpiyachon S, Rattanakarn W. Rice Mutation Breeding for Various Grain Qualities in Thailand. Ministry of Agriculture and Cooperatives [Online] 2011 [cited 2018 December 20] Available from: https://www.fnca.mext.go.jp/english/mb/rice/pdf/8_thailand.pdf

Thitisaksakul M, Tananuwong K, Shoemaker CF, Chun A, Tanadul OUM, Labavitch JM, Beckles DM. Effects of Timing and Severity of Salinity Stress on Rice (Oryza sativa L.) Yield, Grain Composition, and Starch Functionality. J Agric Food Chem 2015; 63(8): 2296-2304.

Waziri A, Kumar P, Purty RS. Saltol QTL and Their Role in Salinity Tolerance in Rice. Austin J Biotechnol Bioeng. 2016; 3(3): 1067.

Yin YG, Kobayashi Y, Sanuki A, Kondo S, Fukuda N, Ezura H et al. Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (Solanum lycopersicum L. cv. 'Micro-Tom') fruits in an ABA- and osmotic stress-independent manner. Journal of experimental botany 2010; 61(2): 563-74.

Zhikang L, Jianlong X. Breeding for Drought and Salt Tolerant Rice (Oryza Sativa L.): Progress and Perspectives. Institute of Crop Sciences/National Laboratory for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences; 2011. p. 1-32.