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# Metabolic profiles and some physiological traits of three rice cultivars differing in salinity tolerance under salinity stress at the germination stage

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Received: June 13, 2023 Accepted: August 03, 2<u>023</u>

Published Online: August 28, 2023

#### Abstract

This study aimed to investigate salinity's impact on the germination process and the metabolic profile of germinated seedlings in three rice cultivars known for their varying levels of salinity tolerance. The seeds of three rice cultivars, KDML105 (saltsensitive), IR29 (salt-sensitive), and Pokkali (salt-tolerant), were germinated in two different treatments: distilled water and a 40 mM NaCl solution. Salinity delayed seed germination and caused root shape abnormalities. The root length of all cultivars was significantly decreased by salinity; however, Pokkali showed the highest root length among these cultivars. Salinity also decreased total soluble sugar, glucose, and fructose content, while starch content was unaffected.  $Na^+/K^+$  ratio in root and shoot was significantly increased in all cultivars; however, the increase was much lower in the tolerance cultivar. Metabolic profiles revealed several biomarkers of the salt stress response among cultivars and between the control and stressed groups. Gammaaminobutyric acid was increased in all cultivars. Citric acid, L-lysine, and L-valine were reduced, while uracil was increased in KDML105. L-lysine, succinic acid, and L-methionine were decreased, while adenine was increased in IR29. Oxalacetic acid, L-proline, and urea increased while melatonin decreased in Pokkali.

**Keywords**: Metabolic profiles, Physiological traits, Rice cultivars, Pokkali, Salinity stress

#### How to cite this:

Jumpa T, Phetcharaburanin J, Suksawat M, Pattanagul K and Pattanagu W. Metabolic profiles and some physiological traits of three rice cultivars differing in salinity tolerance under salinity stress at the germination stage. Asian J. Agric. Biol. 2024(1): 2023088. DOI: <u>https://doi.org/10.35495/ajab.2023.088</u>

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# Introduction

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Rice (*Oryza sativa* L.) serves as the main staple for nearly half of the global population. Nonetheless, the growth and productivity of rice are notably constrained by soil salinity, particularly during its initial growth phase (Rahman et al., 2009). Soil salinity in rice fields is a major problem, especially in Thailand. Salinity stress usually disturbs many aspects of rice, such as physiological, morphological, and ultrastructural changes in cells, the synthesis of biochemicals, and the activation of various other



molecular events (Wang et al., 2013). Salt tolerance, a complex quantitative trait in plants, is mediated through multiple adaptive mechanisms, including ion balance, osmotic adjustment, and antioxidant enzyme collectively contribute activities. which to maintaining  $Na^+/K^+$  homeostasis, accumulating compatible solutes, and enhancing stress tolerance (Fu et al., 2018). The response of rice plants to salt stress is also complicated and depends on various factors such as the duration, the growth stage of the rice plant, rice cultivars, and other related variables (Hussain et al., 2017). The difference in salinity tolerance is the summative result of morphological and physiological changes influenced by changes in metabolites. Therefore, a better understanding of how salinity affects some physiological traits and metabolomics is needed.

The severity of the salinity problem has increased significantly during the past decade. Global climate change and improper cultivation practices lead to larger saline-affected areas, which impacts rice growth and productivity. It is, therefore, essential to understand how salinity stress affects rice seedlings' germination and metabolic profile during emergence. In our research, three different rice cultivars with varying responses to salinity stress were studied at the germination stage. These cultivars were KDML105 (local Thai cultivar sensitive to salt), IR29 (salt-sensitive), and Pokkali (salt-tolerant cultivar). The main objective was to evaluate how metabolite profiling using <sup>1</sup>H-NMR analytical platforms and certain physiological characteristics contributed to these rice varieties' overall salinity stress tolerance. Most studies use a high salinity concentration (approximately 100-150 mM NaCl) for salinity treatment. High salinity level, however, results in severe cellular damage, and many changes in the cell come from the detrimental effects of the stress. Our study used a mild salinity level (40 mM NaCl) to determine the adaptive changes to the stress. The knowledge gained from this study is beneficial for improving rice production and salt-tolerant selection in breeding programs with higher yield potential and constancy across environments, climates, and geographic locations (Amelia et al., 2018).

# **Material and Methods**

# Plant material

Seeds of three rice cultivars (*Oryza sativa* L.), including KDML105 (salt-sensitive, local Thai

cultivar), IR29 (salt-sensitive cultivar), and Pokkali (salt-tolerant cultivar), were used in this study. Approximately 240 seeds per cultivar were soaked with deionized water at room temperature overnight and germinated on the filter paper at ambient temperature. The seeds were germinated in the dark condition for two days and then placed in the tissue culture bottles (8-ounce sizes), 20 seeds per bottle, and grown in the tissue culture room at  $25 \pm 1$  °C with 16/18 day/night photoperiod with a fluorescent light intensity of 2200 lux. Rice seeds of each cultivar were separately added with either 5 mL deionized water (control) or 40 mM NaCl (stress), with six replicates for each treatment. Shoot and root lengths were measured every day. Seedlings were harvested on the 9<sup>th</sup> day because the germination rate was stable. After the final count, speed of germination (SG), final germination percentage (FGP), and germination energy percentage (GEP) using the number of germinated seeds four days after soaking (DAS) were calculated by the formula following Pradheeban et al. (2015).

#### **Physiological traits**

For leaf carbohydrate analyses, 50 mg of fresh rice leaf was extracted with 80% (v/v) ethanol. The supernatant was used for sugar analysis, while the remaining residue was digested to glucose by amyloglucosidase for starch analysis. Total soluble sugars, sucrose, fructose, glucose, and starch were determined according to the previously described method (Pattanagul and Thitisaksakul, 2008). Leaf chlorophyll was extracted with 80% (v/v) acetone and determined according to Arnon (1949). Electrolyte leakage was determined by the method described by Baninasab and Ghobadi (2011).  $Na^+/K^+$  ratio in shoot and root tissues was measured using a flame photometer ( $S_2$ ; Thermo Finnigan, Waltham, MA, USA) (Zhao et al., 2014). Lipid peroxidation (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assays were performed following the methods described by Velikova et al. (2000) and Sergiev et al. (1997). Relative water content was determined according to Turner (1981).

# Metabolic profiling

The leaf of a 9-day-old rice seedling was placed in a preheated oven at 55°C. The dried leaf was ground into a fine powder (400 mg). The powder was immersed in 4 mL of buffer solution (1.5 M KH<sub>2</sub>PO<sub>4</sub>, 2 mM NaN<sub>3</sub>, 1% Trimethylsilylpropanoate; TSP) and sonicated thrice for 15 minutes. After adding two milliliters of

urine buffer, the mixture was vortexed and sonicated for 15 minutes. The solution was centrifuged at 3,500 g ( $4^{\circ}$ C) for 35 minutes. The extraction solution was filtrated by using a filter and transferred to the 5 mm NMR tube. A 400 MHz NMR spectrometer (Bruker, Germany) was used to acquire the <sup>1</sup>H-NMR spectra. The MATLAB software adjusted the phase and baseline of all NMR spectra, with the TSP peak set at 0 ppm. The water peak (4.5-5.0 ppm) was removed. statistical total correlation spectroscopy The (STOCSY) in MATLAB software was used to confirm the correlation between factors among the chemical shift (Phetcharaburanin et al., 2020).

#### Statistical analysis

Physiological traits of each cultivar under both control and stressed conditions were compared using an independent sample T-test conducted with IBM SPSS Statistics 26 software (IBM, Armonk, NY, United States). For the metabolome data, principal component analysis (PCA) was applied to reduce the dimensionality of the variables (Wold et al., 1987). To identify bioactive compounds, orthogonal partial least squares (OPLS) regression analysis was utilized to filter out unwanted variables in the data (Worley and Powers. 2016). The identification and quantification of metabolites were accomplished using the Chenomx NMR analysis software and The Human Metabolome Database (HMDB).

#### Results

#### Seed germination development

Rice seed germination at days 3, 6, and 9 is shown in Figure 1C. The germination analysis revealed that NaCl delayed the seed germination and caused abnormalities of root shape in all cultivars. Salinity stress decreased the root length of all cultivars; however, there was a slight change in shoot length. Among the cultivars, Pokkali performed well by producing the longest root. Although the root length of all cultivars was reduced by salinity, Pokkali still showed the highest root length among other cultivars under stress conditions. On the 9<sup>th</sup> day, the root length of all cultivars decreased significantly compared to the control plants ( $p \le 0.01$ ), while only the shoot length of IR29 was significantly lower than the control ( $p \le 0.01$ ) (Figure 1A, 1B).

Salinity stress did not affect germination energy and final germination. However, the speed of germination was significantly decreased in KDML105 and IR29. Moreover, salinity stress significantly decreased root fresh weight, root dry weight, shoot dry weight of IR29, and shoot fresh weight of KDML105 (Table 1).

#### **Physiological traits**

Starch content was not significantly different between all cultivars' control and stress groups. On the contrary, salinity stress significantly decreased total soluble sugars, glucose, and fructose in all three rice cultivars. Sucrose content in KDML105 and IR29 was not significantly different between the control and stress groups. However, sucrose content in Pokkali was significantly higher when the plant was subjected to stress. Salinity stress did not significantly affect chlorophyll a, chlorophyll b, and total chlorophyll content in all cultivars during the germination stage. Similarly, there was no difference in electrolyte leakage between the control and stressed plants of all three cultivars. Under salinity stress, the relative water content of KDML105 was lower than the control plant, while their levels in IR29 and Pokkali were not significantly different between the control and stressed plants. Salinity did not affect MDA in all cultivars, whereas H<sub>2</sub>O<sub>2</sub> in IR29 was significantly increased when the plant was subjected to stress (Table 2).



Figure-1. Effect of salinity on root length (A), shoot length (B), and morphology (C) of three rice cultivars under control and salinity conditions. Scale bar equal 1 cm. Vertical bars represent standard error (SE). The symbols \* and \*\* represents significant differences (p < 0.05 and p < 0.01, respectively), and "ns" represents not significant between the control and stressed groups in each cultivar. (KD; KDML105, IR; IR29, PK; Pokkali, C; control, S; salinity).

	KD_C	KD_S	IR_C	IR_S	PK_C	PK_S
$\mathbf{D}_{\mathbf{r}}$ and $\mathbf{N}_{\mathbf{r}}^{+}/\mathbf{U}^{+}$	$1.885 \pm$	22.037 ±	2.141 ±	44.717 ±	$1.051 \pm$	$23.607 \pm$
KOOL NA /K	0.122	1.668 **	0.224	3.097 **	0.134	2.343 **
Sheet $N_0^+/V^+$	$0.467 \pm$	6.776 ±	$0.498 \pm$	10.569 ±	$0.292 \pm$	$4.245 \pm$
Shoot Na /K	0.047	0.204 **	0.028	1.570 **	0.062	0.148 **
Starch content	341.51 ±	390.31 ±	$234.27 \pm$	$382.79 \pm$	121.77 ±	$245.69 \pm$
(µmole glucose equivalent g <sup>-1</sup> FW)	29.67	54.46 <sup>ns</sup>	19.14	76.51 <sup>ns</sup>	4.97	64.58 <sup>ns</sup>
Emisters content (umala $a^{-1}EW$ )	$46.30 \pm$	29.47 ±	$57.74 \pm$	20.15 ±	$48.66 \pm$	$27.03 \pm$
Filetose content (µnilole g F W)	0.76	2.95**	2.68	3.09**	4.01	1.58**
Sucross content (umals $a^{-1}EW$ )	$142.62 \pm$	$138.12 \pm$	$153.87 \pm$	$154.02 \pm$	136.16 ±	$161.76 \pm$
Sucrose content (µniore g F w)	7.89	5.26 <sup>ns</sup>	5.48	7.51 <sup>ns</sup>	5.89	7.88*
Glucosa content (umala $a^{-1}EW$ )	357.41 ±	$240.69 \pm$	$448.12 \pm$	213.57 ±	$473.10 \pm$	$303.05 \pm$
Glucose content (µniole g F w)	15.95	36.41*	35.76	13.70**	42.40	11.77**
Total soluble sugar content	$17455.96 \pm$	$12855.08 \pm$	$24163.08 \pm$	$11399.37 \pm$	$24057.54 \pm$	$17190.64 \pm$
(µmole g <sup>-1</sup> FW)	2157.39	1491.98*	1392.92	968.91**	2336.74	2031.20**
Chlorophyll a content (ug $g^{-1}EW$ )	438.33 ±	$446.22 \pm$	$451.00 \pm$	$581.92 \pm$	$304.90 \pm$	$331.86 \pm$
Children $u$ content ( $\mu g g F W$ )	57.51	56.75 <sup>ns</sup>	77.09	57.57 <sup>ns</sup>	47.05	45.82 <sup>ns</sup>
Chlorophyll <i>h</i> contant (ug $g^{-1}EW$ )	$167.60 \pm$	$146.96 \pm$	$134.72 \pm$	$199.39 \pm$	$153.34 \pm$	$109.88 \pm$
Chiorophyn <i>b</i> content (µg g Fw)	73.07	56.72 <sup>ns</sup>	65.87	33.94 <sup>ns</sup>	85.91	66.40 <sup>ns</sup>
Total chlorophyll content	547.51 ±	554.01 ±	$530.30 \pm$	$641.70 \pm$	$458.12 \pm$	$354.18 \pm$
$(\mu g g^{-1} FW)$	63.29	87.86 <sup>ns</sup>	46.98	82.00 <sup>ns</sup>	90.56	63.62 <sup>ns</sup>
Flootrolute lookage (%)	$37.97 \pm$	45.91 ±	$22.26 \pm$	$26.06 \pm$	$25.33 \pm$	$29.44 \pm$
Electrolyte leakage (%)	5.31	6.46 <sup>ns</sup>	2.15	2.19 <sup>ns</sup>	2.19	1.71 <sup>ns</sup>
<b>P</b> olotive water content $(0/)$	83.94 ±	81.43 ±	$78.99 \pm$	$78.23 \pm$	$80.41 \pm$	$86.22 \pm$
Relative water content (%)	0.36	0.91*	2.47	3.15 <sup>ns</sup>	3.46	1.53 <sup>ns</sup>
$MDA$ (umplo $a^{-1}EW$ )	$0.0094 \pm$	$0.0083 \pm$	$0.0092 \pm$	$0.0096 \pm$	$0.0045 \pm$	$0.0045 \pm$
MDA (µiiole g Fw)	0.0020	$0.0006^{ns}$	0.0002	0.0015 <sup>ns</sup>	0.0016	0.0007 <sup>ns</sup>
$\mathbf{U} \mathbf{O}$ (uncless <sup>-1</sup> $\mathbf{E}$ <b>V</b> )	$1059.07 \pm$	1071.34 ±	$663.06 \pm$	$879.67 \pm$	$595.95 \pm$	$505.47 \pm$
$\Pi_2 O_2$ (µniole g FW)	107.73	161.02 <sup>ns</sup>	48.58	69.76*	137.04	91.21 <sup>ns</sup>

Table-1. Germination parameters of three rice cultivars at the 9-day-old stage under salinity stress (KD; KDML105, IR; IR29, PK; Pokkali, C; control, S; salinity).

Data are the mean  $\pm$  SE (n = 6). The symbols \* and \*\* represents significant differences (p < 0.05 and p < 0.01, respectively), and "ns" represents not significant between the control and stressed groups in each cultivar.

#### **Metabolic profiles**

In this study, untargeted metabolomics using <sup>1</sup>H-NMR was employed to identify metabolite biomarkers reflecting salt stress responses in different rice cultivars, and the combination of PCA and PLS-DA analysis was used to improve model predictability, accommodate outliers, and enhance group discrimination, leading to the successful separation of each cultivar and clear differentiation between stress-treated and control groups. The spectral data obtained from three cultivars with two conditions were used to construct the PCA model as evaluated by its degree of fit  $(R^2X)$  and predictability  $(Q^2)$ , accounting for 65.1 % and 44.4 %, respectively. The tightest clustering of KDML105 was noticeable, while IR29 and Pokkali showed a larger degree of intra-variation. IR29 and KDML105 were separated along the first principal component (PC<sub>1</sub>,  $R^2X =$ 23%), while the second principal component ( $PC_2$ ,

 $R^2X = 16.4\%$ ) determined the metabolic differences between Pokkali and the others (Figure 2A).



Figure-2. PCA score plot, each marker on the score plot refers to single sample with  $R^2X$  (cumulative) = 65.1 % and  $Q^2$  (cumulative) = 44.4% (A) and PLS-DA plots, each marker on the score plot refers to a single sample with  $R^2X$  (cumulative) = 58.7%,  $R^2Y$  (cumulative) = 67.3% and  $Q^2$  (cumulative) = 37.4% (B) of seed germination metabolome of rice cultivars with control and stress conditions (n=6 each).

Partial least squares projections to latent structures



discriminant analysis (PLS-DA) was performed on metabolome data to improve model predictability and strengthen group discrimination. In the PLS-DA analysis, all six sample sets, including KDML105 (control), KDML105 (stress), IR29 (control), IR29 (stress), Pokkali (control), and Pokkali (stress), were separated with 37.4% predictive capability. Pokkali was separated from the others along the PLS-DA component 2, explaining the major variation (15.7%). In addition, the salinity-stressed sample was discriminated from the control groups of each cultivar along the PLS-DA component 1 (21.6%). Moreover, PLS-DA component 1 demonstrated the separation between KDML105 and IR29 (Figure 2B).

Table-3. Metabolite names of three rice cultivars under control and salinity conditions at the germination stage.

KD	IR	РК		Metabolite	KD	IR	PK	No.	Metabolite
			1	L-valine				18	sarcosine
			2	methyl malonate				19	indole acetaldehyde
			3	L-lactate				20	xanthine
			4	lysine				21	oxaloacetate
			5	isoleucine				22	glycyl-glycine
			6	L-proline				23	melatonin
			7	acetoin				24	pantothenate
			8	glutarate				25	choline
			9	succinate				26	3- chlorotyrosine
			10	$\gamma$ -aminobutyrate				27	urea
			11	NADPH				28	guanosine
			12	acetoacetate				29	tartarate
			13	trimethyl pyrazine				30	L-carnitine
			14	methionine				31	erythrose
			15	citrate				32	maltose
			16	trimethylamine				33	uracil
			17	adenine					

KD; KDML105, IR; IR29, PK; Pokkali. The red, gray, and blue colors indicate increased, unchanged, and decreased metabolites.

The discriminatory metabolites of each treatment compared with the control group were investigated through OPLS-DA analyses, Statistical Total Correlation Spectroscopy (STOCSY) matched, and the lists of all metabolic shifts found in the rice germination stage. Our findings exhibited that under salinity stress, KDML105 possessed significantly decreased levels of L-valine, lysine, isoleucine, citrate, indole acetaldehyde, melatonin, pantothenate, and maltose, while, L-lactate, L-proline, acetoin, 4aminobutyrate, NADPH, sarcosine, 3-chlorotyrosine, urea, guanosine, L-carnitine, ervthrose, and uracil were remarkably increased (Figure 3A). IR29 grown under salinity stress conditions showed a significant decrease in the levels of L-valine, lysine, succinate, acetoacetate, methionine, trimethylamine, melatonin, and choline, accompanied by significantly increased levels of methylmalonate, L-lactate, isoleucine, Lproline, acetoin, glutarate, 4-aminobutyrate, trimethyl pyrazine, adenine, xanthine, glycyl-glycine, and tartarate (Figure 3B). Under salinity stress, Pokkali demonstrated a decreased relative concentration of only melatonin, while L-valine, methylmalonate, L-4-aminobutyrate, lactate, L-proline, sarcosine, oxaloacetate, 3-chlorotyrosine, urea, and L-carnitine were increased (Figure 3C). The predictive component of KDML105, IR29, and Pokkali is  $R^2X$  of 44.6%, 46.7%, and 52.7%, respectively, and  $Q^2Y$  of 95.5%, 89.3%, and 88.0%, respectively, with the metabolite name in Table 3.

The pathway analysis was performed to elucidate each cultivar's metabolic phenoconversion to understand further the metabolic adaptation of the three cultivars grown under salinity stress conditions. In addition to the physiological adaptation, our findings showed that all cultivars responded to the salinity stress differently. In KDML105, pantothenate and CoA biosynthesis was significantly affected ( $p \le 0.05$ ) in response to salinity stress conditions. IR29 grown under salinity stress conditions exhibited five significant impacted metabolic pathways ( $p \le 0.05$ ), including aminoacyl-tRNA biosynthesis; butanoate metabolism; valine, leucine, and isoleucine degradation; valine, aspartate, and glutamate metabolism.

Four metabolic pathways showed significance ( $p \le 0.05$ ), including pyruvate metabolism; alanine, aspartate, and glutamate metabolism; glycolysis/ gluconeogenesis; arginine and proline metabolism.

Figure 4 illustrates the simple metabolic pathways involved in the salinity response of three rice cultivars with the bars represented. Metabolites highlighted in this figure were altered when exposed to salinity conditions with a significantly high pathway impact. These are oxaloacetate, lactate, melatonin, indole acetaldehyde, succinate, gammaamino butyrate, L-valine, isoleucine, acetoacetate, Lproline, methylmalonate, uracil, and pantothenate.



Figure-3. O-PLS-DA loadings plot based on <sup>1</sup>H-NMR metabolic profiles of KDML105, IR29, and Pokkali under stress condition and their control counterparts on the 9<sup>th</sup> day. The color of <sup>1</sup>H-NMR resonances in the O-PLS-DA loading plot signifies the contribution of the metabolites towards class segregation between either the control or stress group with the minor significance of blue and the highest significance of red color. The upward orientation of the peaks denotes a relatively higher concentration of the corresponding metabolites in the stress group, and the downward direction of the peaks indicates a relatively higher concentration of the related metabolites in the control group.



Figure-4. The schematic metabolic pathways involved in the salinity response of three rice cultivars at the germination stage modified from the KEGG pathway. KD; KDML105, IR; IR29, PK; Pokkali. The red, gray, and blue colors indicate increased, unchanged, and decreased metabolites.

# Discussion

Salinity stress delays seed germination in the sensitive cultivars of rice, reducing the speed of germination, root length, and root biomass. Reduced root growth may contribute to lower relative water content and shoot seedling growth. The sensitive cultivars exhibited elevated  $Na^+/K^+$  ratios in both their roots and shoots, resulting in higher levels of electrolyte leakage, MDA,  $H_2O_2$ , and reduction in

sugar contents.\_Seed germination is an early and crucial process for rice cultivation. It determines when plants enter agricultural environments and can relatively regulate their seedling growth and yield. In this study, three rice cultivars differing in salinity tolerance were compared with a focus on seed germination parameters. It is found that salinity condition causes abnormalities of root shape. NaCl may directly affect cell division and growth, as evidenced by the reduction of radicle elongation and



morphological alterations, similar to cadmium stress (He et al., 2008). Salinity stress did not affect all cultivars' germination energy and final energy. However, the speed of germination was considerably affected when the rice seed was exposed to salinity. In the tolerant cultivar, the speed of germination was slightly decreased compared to the sensitive cultivar, which showed a dramatic decrease in the speed of germination. A similar conclusion was reported by Rahman et al. (2009), who found that NaCl up to 0.3% (approximately 50 mM) postponed germination but did not reduce the final germination percentage. This study highlights the differential responses of rice cultivars to salinity stress. While the salt-tolerant can maintain shoot and root biomass, salinity stress heavily reduces the shoot and root growth of the sensitive cultivar. Soil salinity triggers the responses in roots that influence the efficiency of water uptake and ion elimination because of changes in the roots' biochemical. molecular. and physiological interactions (Yuenyong et al., 2018). The adaptability of a plant to different soil conditions is highly dependent on its root system architecture, which is primarily regulated by cell division and elongation in primary and lateral roots, enabling water and mineral transport as well as communication of essential signals within the plant (Dorlodot et al., 2007). Under soil salinity, the ability to sustain root growth is considered a key determinant for successful growth under salinity conditions of the tolerant cultivar.

Common salt tolerant mechanisms in plants are ion compatible solute accumulation. balance and Maintaining a low cytosolic Na<sup>+</sup>/K<sup>+</sup> ratio is crucial for reducing oxidative damage induced by salinity stress, as elevated Na<sup>+</sup> influx and inhibited K<sup>+</sup> uptake disrupt Na<sup>+</sup>/K<sup>+</sup> homeostasis, ultimately resulting in detrimental consequences for plant survival (Zhu, 2003). The present study found that the  $Na^+/K^+$  ratio was significantly increased in both the shoot and root of all cultivars. However, the  $Na^+/K^+$  ratio in the sensitive cultivar is much higher than those in the tolerance cultivar. The difference in the  $Na^+/K^+$  ratio between treatments of each cultivar suggests that Na<sup>+</sup> exclusion from leaf tissues plays a vital role in the salt tolerance of rice by preserving the optimal  $Na^+/K^+$  ratio. Therefore, Pokkali can maintain a low  $Na^{+}/K^{+}$  ratio and displays a fast growth rate under salinity conditions, making it more tolerant to stress (Walia et al., 2005). Compatible solute accumulation serves as an abiotic stress-tolerant mechanism, as these compounds, including total soluble sugars such

as glucose and fructose, increase under saline stress conditions and act as indicators of the common tolerance mechanism to salinity stress in plants (Singh et al., 2015). The inhibition of rice seed germination under salt stress, as indicated by decreased a-amylase activity and reduced bioactive gibberellin levels, can be attributed to the accumulation of sucrose and other carbohydrates as compatible solutes in salt-tolerant rice cultivars (Liu et al., 2018). Salt-induced damage includes the elevation of reactive oxygen species (ROS) levels, such as superoxide, hydrogen peroxide, and hydroxyl radical, which can cause oxidative stress by peroxidation of membrane lipids, protein degradation, and nucleic acid mutation, highlighting the importance of intracellular antioxidants in neutralizing ROS (Czégény et al., 2014). MDA, a marker of oxidative stress resulting from lipid peroxidation, is known to increase due to the overproduction of free radicals and has the potential to cause DNA damage; however, this study observed no lipid peroxidation in any cultivar during the mild salinity stress in the rice seed germination stage, while increased  $H_2O_2$  levels in the sensitive cultivar suggest a potential for lipid peroxidation and DNA damage in later stages under salinity stress (Ahmad et al., 2010). While many studies have used photosynthetic pigment content as an indicator of the abiotic stress response, this study found no significant change in chlorophyll content among all cultivars under salinity conditions during the rice germination stage; however, other studies have reported a reduction in chlorophyll content with increasing salt concentration, especially in saltsensitive cultivars, indicating that chlorophyll content in the germination stage may be constrained due to the presence of an endosperm for nutrient storage and the non-essential role of photosynthesis during this stage (He and Yang, 2013).

KDML105, IR29, and Pokkali metabolome analysis revealed distinct metabolic profiles for each cultivar. These differences suggest unique metabolic changes occurring in each cultivar, which can be attributed to genetic factors and the expression of specific genes (Wang et al., 2018). In all cultivars, gammaaminobutyrate (GABA) exhibited the maximum potential functional relationship with other metabolites in the network and showed a significant increase under mild salinity conditions during rice seed germination. The positive regulation of the GABA shunt and associated pathways improve plant



tolerance toward abiotic stress (Bao et al., 2015). In KDML105 and IR29, L-lysine decreased in response to salinity stress, making it a promising candidate for supporting callus formation and potentially increasing osmotic pressure to facilitate water uptake from neighboring cells (Alreda et al., 2020). Proline accumulation increased across all cultivars, indicating its common physiological response to various stresses. Proline helps mitigate osmotic stress and acts as an antioxidant to protect cells against abiotic stressors in salt-tolerant rice under NaCl conditions (Kibria et al., 2017). Melatonin levels decreased under salinity conditions in all cultivars but exhibited the maximum interaction in Pokkali. Melatonin is a multifunctional signaling molecule that improves antioxidant activities and acts as a defense against adverse environmental conditions (Debnath et al., 2019). It also regulates seminal root length and root growth after germination in rice plants (Back and Park, 2012).

Under salinity stress, our study observed an increase in oxaloacetate (OAA), a crucial intermediate in the citrate cycle for ATP production in cellular respiration, which exhibited strong associations with other metabolites in Pokkali. The result suggests that elevated OAA levels in response to stress may be directed towards amino acid synthesis for protein formation and meeting the heightened energy demands of the cell (Berg et al., 2002). Urea levels were increased in Pokkali, in which a similar result was observed in barley under salinity conditions (Derakhshani et al., 2020). This study identified polyamines (spermine and spermidine), propionate, and uracil as precursors of  $\beta$ -alanine in plants, which can be further converted into the osmoprotective compound  $\beta$ -alanine betaine in certain species and into the antioxidant homoglutathione (Parthasarathy et al., 2019). KDML105 exhibited decreased levels of citrate and L-valine. Significant reductions in citrate, alpha-ketoglutarate, fumarate, malate, and succinate in the TCA cycle, suggest impaired energy production due to high salinity stress (Wu et al., 2013). Succinate, a key intermediate in the ATP and citrate pathways, is crucial in energy production and regulating the mitochondrial TCA cycle. Its elevated levels have been associated with increased tolerance to abiotic stress, suggesting its potential for enhancing energy generation through efficiently utilizing the TCA cycle under water-limited conditions (Ullah et al., 2017).

Salinity stress typically induces the accumulation of

methionine in plants, resulting in significant morphological, physiological, and biochemical changes across various plant organs (Michaletti et al., 2018). Muscolo et al. (2015) found increased levels of methionine, isoleucine, valine, arginine, proline, and histidine in all genotypes, contributing to the production of osmoprotectants and enhancing stress tolerance. Threonine and methionine serve as substrates for isoleucine synthesis, and their synthesis and breakdown under different conditions developmental and environmental influence the availability of isoleucine. Accumulation of specific amino acids is believed to contribute to plant stress tolerance as osmolytes (Joshi et al., 2010). In this study, however, succinate and L-methionine are reduced in IR29. Adenine, a significant source of organic nitrogen in culture media, was up-regulated along with guanine, indicating the activation of a protective mechanism to safeguard nucleotides, as observed in soybean (Michaletti et al., 2018). In IR29, adenine levels were increased, consistent with the findings of Mirnezhad et al. (2010), who utilized NMR metabolomics to investigate host plant resistance to thrips in wild and cultivated tomatoes, demonstrating that wild tomatoes exhibited significant resistance to thrips compared to the grown tomatoes.

# Conclusion

The salt-tolerant nature of Pokkali is possibly resulting from its efficiency in producing higher root length, maintaining the speed of germination and relative water content, and accumulation of some osmoprotectants under stress conditions. Moreover, each rice cultivar responds differently to salinity stress regarding physiology and metabolites. KDML105 shows similar responses to IR29, confirming their salinity stress sensitivity. Pokkali, on the other hand, expresses a different set of metabolic profiles which explain its salt tolerance.

# Acknowledgment

Many thanks to the Department of Biology, Faculty of Science, Khon Kaen University, Thailand for providing the laboratory facilities and greenhouse space.



# Disclaimer: None.

Conflict of Interest: None.

**Source of Funding:** This study was funded under Development and Promotion of Science and Technology Talents Project (DPST) and KKU Research and Graduate Affairs (Project No. RP64-11-001), Thailand.

### **Contribution of Authors**

Jumpa T: Contributed in data collection & analysis, writing original draft of article and final proofreading Phetcharaburanin J & Pattanagu W: Conceived idea and developed research methodology, collected data, final proofreading and funding acquisition

Suksawat M: Developed research methodology and collected data

Pattanagul K: Contributed in data validation and final proofreading

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