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Metabolite profiles of two cultivars of Thai rice *Oryza sativa* L. that are different in salt tolerance

Kwankao Karnpakdee[a,*]

[a]*Department of Biochemistry, Faculty of Science, Chulalongkorn University, Thailand*

[*]Corresponding author. Tel: +66816581336
E-mail address: kwankao.film@gmail.com

Abstract. Rice (*Oryza sativa* L.) is an important crop in Thailand. However, its production yield has been affected by salinity stress. In order to understand the mechanisms underlying salinity stress response in rice, we applied the metabolomics as a tool to compare metabolite profiles between the moderately salt-sensitive 'KDML 105' and the salt-tolerant rice 'UBN 02123-50R-B-2' under normal condition. Two-week-old leaves and roots were collected. The metabolite profiles of both cultivars were then profiled using GC-MS and LC-MS. Principal component analysis (PCA) indicated the different metabolite profiles between the salt-sensitive and the salt-tolerant cultivars. The results showed the higher accumulation of stress-related metabolites in the salt-tolerant cultivar including galacturonic acid in both leaves and roots, as well as ferulic acid only in roots. These up-accumulated metabolites might serve a proactive role against salinity stress. We are now investigating the metabolic responses of these two cultivars under salinity stress that could provide a better understanding in metabolic responses to salinity stress.

Keywords: *Oryza sativa* L.; metabolomics; salinity stress; salt-tolerance

1. Introduction

Biological process is very crucial mechanism for growth and development of plants under harsh conditions. The understanding of such process is not only reveal plant defense mechanisms, but also being the primitive data for further study. The stress conditions are the common faced-conditions of plant worldwide. It could affected plants in many way such as dehydration, ion toxicity and oxidative stress. More importantly, they could also change metabolic flux and nutrient balances of plants (Tester and Davenport, 2003). For example, environmental stresses have been reported the changes in cellular metabolic flux, especially energy storage metabolites and the compatible solutes. In response to defend stresses, plants could adapt themselves by accumulating the primary and secondary metabolites. Recently, the responses to salinity stress were studied in wild barley (*Hordeum spontaneum*) and cultivated barley *(Hordeum vulgare).* The result exhibited the difference in metabolite levels. They showed the increase in the levels of sugars and amino acids, as decrease in the intermediate flux of tricarboxylic acid cycle (Wu et al, 2013).

The metabolomics is a practical tool that has widely been applied for studying the metabolite flux. This is because it could potentially identified metabolites presented in one or various biological conditions, and could combine the statistical methods with high resolution techniques.

There are various forms of stresses that could attack plants. Reportedly, the salinity causes the main abiotic stress that could undoubtedly effect growth of rice. However, the unpleasure environmental conditions might not cause negative effect for all (Munns and Tester, 2008). In this study, we therefore estimated the proactive action against salinity stress in Thai rice *O. sativa* cv. KDML 105 and the salt-tolerant rice 'UBN 02123-50R-B-2'(UBN) under normal condition, using the metabolomic strategies. We grew the rice under control condition to lessen the effects of conventional changes of global flux. The leaves and roots of rice were sampled for analysis by GC-MS and LC-MS. The metabolite profiles were then constructed by PCA. The metabolite profiles of KDML 105 and UBN were compared to the defined metabolite markers to prove the action as proactive against salinity stress. The obtianed metabolite profiles from normal condition are subsequently integrated with the ones obtained from salinity stress conditions.

2. Experimentals

2.1 Hydroponic culture

The wild type rice cultivar 'KDML 105' and the salt-tolerant rice 'UBN 02123-50R-B-2' (UBN) seeds were sterilized in 35% sodium hypochloride and washed several times with deionized water. Seeds of two cultivars were then soaked in deionized water until germinated. The germinated seeds were transferred into the Yoshida nutritional medium (Yoshida et. al., 1976). They were grown under the control growth condition as 16/8 light cycle, 25˚C, the light intensity at 7,000 lux, for 14 days.

2.2 Metabolite profiling analysis

The six biological replications were collected from each condition. 100 mg of fresh samples were extracted, derivatized and analysed by using GC-MS and LC-MS according to Kusano et al., 2011 in collaboration with the RIKEN Center for Sustainability Resource Science, Japan. The raw data were manipulated with SIMCA software (SIMCA, http://www.umetrics.com/), applying the Partial Least Squares Discriminant *Analysis* (*PLS-DA*) model combined with Student's T test (T-test) (P<0.05).

3. Results and discussions

3.1 The PCA analysis under normal condition

The analyzed GC-MS and LC-MS data were indicated by PCA (Fig 1). The models were constructed from the group of GC-MS (A) and LC-MS (B) analysis according to PLS-DA. The clusters illustrated the separation of the metabolite groups between two cultivars which showed similar pattern in both roots and leaves tissues. The results suggested that the metabolite profile of KDML 105 is inherently different one of UBN in normal growth condition. Thus, they might participate in different responses to salinity stress. This could depend on the variation of salinity tolerances of the monocotyledonous species as their proactive action (Munns and Tester, 2008).

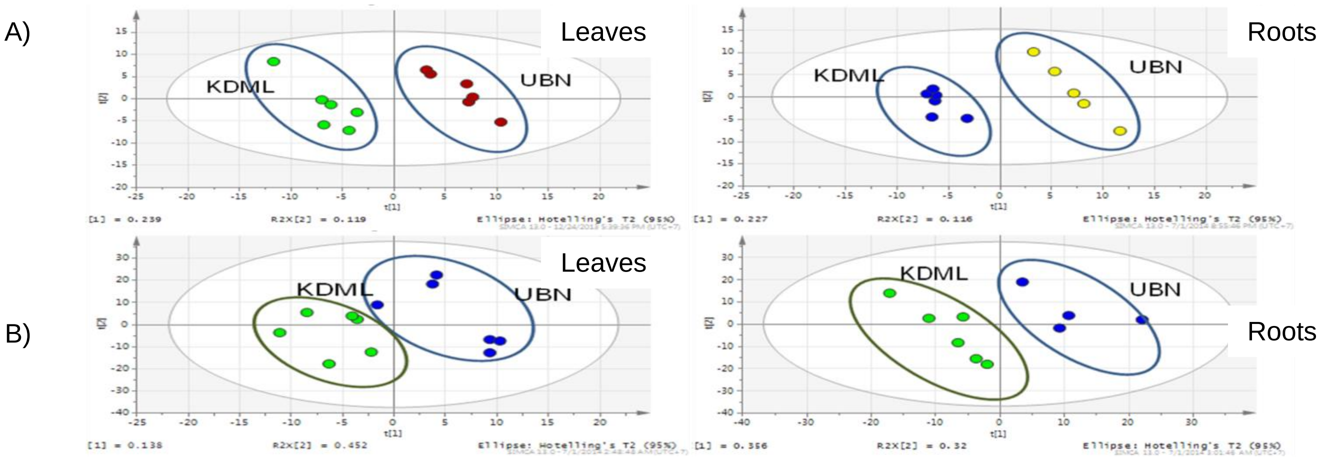

Fig. 1. PCA of metabolites from the dataset of GC-MS (A) and LC-MS (B) of KDML 105 and UBN under normal condition

3.2 Metabolite analysis in normal condition

To evaluate how salinity stress affects metabolic responses, we studied the accumulated levels of metabolites normally existed in normal growth condition. The metabolite data from two cultivars were performed using Student t-test (p<0.05) to calculate the significantly difference in quantitative data of metabolites between KDML 105 and UBN. Due to UBN playing roles as the salt-tolerance rice, its metabolites are represented as the hypothesized case to understand the metabolic responses to salinity stress. The results of UBN from GC-MS data revealed predominantly high accumulation of galacturonic acid (Table 1) which involves in ascorbic acid biosynthesis pathway (Valpuesta and Botella, 2004). Ascorbic acid in plants has been reported as a bioactive compound with often claimed to be an important antioxidant agent (Aruoma, 2003) that could protect cells from reactive oxygen species (ROS). Therefore, the accumulation of galacturonic acid might be served as a precursor to the ascorbic acid biosynthesis in responses to salinity stress. Galacturonic acid also occurred in high accumulation in roots as showed in Table 2. Additionally, the levels of several amino acids and compatible solutes were high accumulation in UBN. There are tyrosine, proline, inositol and spermidine. These metabolites have been reported as compatible solutes responded to salinity stress by accumulating in the cytosol and organelles to balance the osmotic pressure (Munns and Tester, 2008). The various accumulated metabolites in roots might cause proactive action due to roots directly contacting with soil and growth solutions.

We also found the accumulation of tricin 7-O-beta-D-glucopyranoside, isoscoparin 2''-(6-(E)-ferulylglucoside) and neocarlinoside in leaves, and ferulic acid in roots from LC-MS data analysis (Table 3). Accordingly, these metabolites in leaves and roots were correlated with the results from PLS-DA score plot. Generally, ferulic acid is an important structural component in the plant cell wall (Chi et al, 2013). Also, the antioxidant activity of ferulic acid and its ester has been in good agreement with their radical scavenging activities (Kikuzaki et al, 2002).

Table 1. The accumulation of metabolites in leaves under normal condition, detected by GC-MS

	Category	Metabolite	Accumulated level		Significant
			KDML	UBN	
Leaves	Sugar & Polyols	Raffinose	2.125184	0.470548	*
	TCA cycle	Fumarate	1.527751	0.654557	**
	Others	Beta-alanine	2.796643	0.357572	***
		Galacturonic acid	0.219175	4.562563	*
		Glutaric acid	2.119157	0.471886	*
		5-hydroxytryptamine	3.835031	0.260754	*
		Lumichrome	2.68932	0.371841	**
		Nicotianamine	1.683333	0.59406	***

Table 2. The accumulation of metabolites in roots under normal condition, detected by GC-MS

	Category	Metabolite	Accumulated level		Significant
			KDML	UBN	
Roots	TCA cycle	Citrate	2.3	0.43	*
	Amino acids	Cysteine	0.58	1.72	**
		Proline	0.59	1.69	*
		Tyrosine	0.48	2.08	***
	Others	Galacturonic acid	0.27	3.72	**
		Glyceric acid	1.9	0.53	**
		Inositol	0.66	1.52	*
		Lumichrome	5.99	0.17	***
		Nicotianamine	0.57	1.75	*
		Ornithine	1.75	0.57	*
		Pentasiloxane, dodecamethyl	0.47	2.15	***
		Phosphoric acid monomethyl ester	5.4	0.18	**
		Piperidin-2-one, 3-amino-	6.73	0.15	*
		Spermidine	0.67	1.49	***

Table 3. The accumulation of metabolites in KDML 105 and UBN under normal condition, detected by LC-MS

Tissue	Metabolite	Retention time	m/z	Accumulated level		Significant
				KDML	UBN	
Leave	Tricin 7-O-beta-D-glucopyranoside	4.5058	491.1186	0.91	1.09	*
	Schaftoside, Neoschaftoside	3.5164	563.1396	0.89	1.12	**
	Neocarlinoside, Carlinoside	3.2221	579.1354	0.70	1.45	**
	Isoscoparin 2"-(6-(E)-ferulylglucoside)	4.1396	799.2076	0.81	1.24	*
	Neocarlinoside, Carlinoside	3.2215	581.1511	0.81	1.23	*
Root	Ferulic acid	3.2277	193.0506	0.49	2.01	*

The accumulation of ferulic acid could be hinted the possible action of proactive coping stress responses in UBN. Moreover, we also found the various un-annotated metabolites detected by LC-MS (data not shown) which significantly distinguished between KDML 105 and UBN in normal growth condition. We are also investigating this as our future studies.

4. Conclusions

Our metabolite profiles could raise a new perspective on the salinity responses of two Thai rice cultivars. The differences in the accumulated metabolite levels were conducted by GC-MS and LC-MS. In normal growth condition, UBN roots exhibited higher accumulation in amino acids, organic acids and other bioactive metabolites that might act as osmoprotectants, or might lead to encounter more stresses. In contrast, KDML leaves showed higher accumulation in raffinose, 5-hydroxytryptamine and lumichrome. Hence, it could be concluded that both rice cultivars inherently present metabolite levels under normal growth condition. These metabolites are able to define as the metabolite markers that might be affected by salinity. We are now studying for the integration of the stress-related metabolites data.

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6. Conflict of interest: none

7. References

Aruoma,O. 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Research. 9, 523-534.

Chi, WC., Chen, YA., Hsiung, YC., Fu, SF., Chou, CH., Trinh, NN., Chen, YC., and Huang, HJ. 2013. Autotoxicity mechanism of Oryza sativa: transcriptome response in rice roots exposed to ferulic acid. BMC Genomics. 14, 1471-2168.

Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K., and Taniguchi, H. 2002. Antioxidant Properties of Ferulic Acid and Its Related Compounds. Journal of Agricultural and Food Chemistry. 50, 2161-2168.

Kusano, M. , Tabuchi, M., Fukushima, A., Funayama, K., Diaz, C., Kobayashi, M., Hayashi, N., Tsuchiya, YN., Takahashi, H., Kamata, A., Yamaya, T., and Saito, K. 2011. Metabolomics data reveal a crucial role of cytosolic glutamine synthetase 1;1 in coordinating metabolic balance in rice. The Plant Journal. 66, 456-466.

Munns, R. and Tester, M. 2003. Mechanisms of Salinity Tolerance. Annual Review of Plant Biology. 59, 651-681.

Tester, M and Davenport, R. 2003. Na^+ Tolerance and Na^+ Transport in Higher Plants. Annual of Botany. 91, 503-527.

Valpuesta, V. and Botella, M. 2004. Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. Plant Science. 9, 573-577.

Wu, D., Cai, S. ,Chen, M., Ye, L., Chen, Z., Zhang, H., Dai, F., Wu, F., and Zhang, G. 2013. Tissue Metabolic Responses to Salt Stress in Wild and Cultivated Barley. PLOS One. 8, 1-11.

Yoshida, S., Forno, A. D., Cock, H. J., and Gomez, A. K. 1976. Laboratory Manual for Physiological Studies of Rice. Los Baňos, Laguna, Philippines, pp. 61-66.