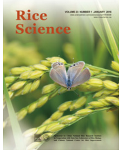
Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2016, 23(1): 9−21



Differential Proteins Expressed in Rice Leaves and Grains in Response to Salinity and Exogenous Spermidine Treatments

Paweena SALEETHONG[1], Sittiruk ROYTRAKUL[2], Kanlaya KONG-NGERN[3], Piyada THEERAKULPISUT[3]

(*[1]Rajamangala University of Technology, Isann Surin Campus, Surin 32000, Thailand; [2]National Center for Genetic Engineering and Biotechnology, Pathum Thani 12120, Thailand; [3]Salt-tolerant Rice Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*)

Abstract: Exogenous application of spermidine (Spd) has been reported to modulate physiological processes and alleviate salt-induced damage to growth and productivity of several plants including rice. Employing a proteomic approach, we aimed at identifying rice leaf and grain proteins differentially expressing under salt stress, and in response to Spd prior to NaCl treatment. A total of 9 and 20 differentially expressed protein spots were identified in the leaves of salt-tolerant (Pokkali) and salt-sensitive (KDML105) rice cultivars, respectively. Differential proteins common to both cultivars included a photosynthetic light reaction protein (oxygen-evolving complex protein 1), enzymes of Calvin cycle and glycolysis (fructose-bisphosphate aldolase and triose-phosphate isomerase), malate dehydrogenase, superoxide dismutase and a hypothetical protein (OsI_18213). Most proteins were present at higher intensities in Pokkali leaves. The photosynthetic oxygen-evolving enhancer protein 2 was detected only in Pokkali and was up-regulated by salt-stress and further enhanced by Spd treatment. All three spots identified as superoxide dismutase in KDML105 were up-regulated by NaCl but down-regulated when treated with Spd prior to NaCl, indicating that Spd acted directly as antioxidants. Important differential stress proteins detected in mature grains of both rice cultivars were late embryogenesis abundant proteins with protective roles and an antioxidant protein, 1-Cys-peroxiredoxin. Higher salt tolerance of Pokkali partly resulted from higher intensities and more responsiveness of the proteins relating to photosynthesis light reactions, energy metabolism, antioxidant enzymes in the leaves, and stress proteins with protective roles in the grains.

Key words: *Oryza sativa* L.; proteomics; salt stress; spermidine

Salt stress is one of the major abiotic stresses prevalent in agricultural areas worldwide. Excess salts in soils inhibit plant growth and productivity for two main reasons. The first one is an osmotic effect that reduces the capacity of plant water uptake and causes a slow plant growth, and the second is the toxic effect due to the entry of salts that cause tissue damage of the leaves (Zhu, 2002; Khan et al, 2007). Rice (*Oryza sativa* L.) 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 3.0 dS/m (Grattan et al, 2002). Salinity affects all stages of growth and development of rice, particularly early seedling, pollination and fertilization stages (Lutts et al, 1995; Gregorio et al, 1997). Different varieties of rice express widely different levels of salt tolerance (Gregorio et al, 2002; Zeng et al, 2003; Kanawapee et al, 2012). Mechanisms for increasing tolerance to salt stress involve the production of stress proteins, the accumulation of compatible solutes (sugars, amino and organic acids, betaines and polyamines), and the expression of different sets of genes (Jimenez-Bremont et al, 2007). Plant growth regulators including polyamines (PAs) and other groups of chemicals have been used to treat rice plants exogenously at various growth stages to increase salt tolerance by alleviating

Received: 15 April 2015; **Accepted:** 30 July 2015

Corresponding author: Piyada THEERAKULPISUT (piythe@kku.ac.th)

Peer review under responsibility of China National Rice Research Institute
http://dx.doi.org/10.1016/j.rsci.2016.01.002

salt-induced damages and lead to improved growth and productivity (Roychoudhury et al, 2011; Plaut et al, 2013).

In plants, PAs are known to play regulatory roles in various physiological events, such as cell division, root initiation, flower development, and fruit ripening and senescence, and also have beneficial effects on abiotic stress responses (Alcazar et al, 2006). For salinity, exogenous PAs have been documented to reduce salinity stress-induced damages including the accumulation of Na^+, loss of K^+, chlorophyll degradation, inhibition of photochemical reaction of photosynthesis, reactive oxygen species (ROS) accumulation, lipid peroxidation and membrane damage, and the enhancement of activity of antioxidative enzymes and non-enzymatic antioxidants and compatible osmolytes (Chattopadhyay et al, 2002; Roy et al, 2005; Roychoudhury et al, 2011). Exogenous spermidine (Spd) reverses the inhibitory effects of salt stress in both salt-tolerant (Pokkali) and salt-sensitive (KDML105) rice cultivars (Saleethong et al, 2011). Salt-induced damage including chlorophyll loss, membrane leakage, ROS (H_2O_2) accumulation, lipid peroxidation, Na^+ accumulation and K^+ reduction were all alleviated by Spd pretreatment, resulting in improved growth performance (Zhu et al, 2006; Duan et al, 2008; Saleethong et al, 2011). In reproductive stage, salinity treatment imposed during late booting stage to maturity stage resulted in significant reductions in yield and yield-related parameters of both Pokkali and KDML105, and pretreatment by foliar spraying of Spd to rice plants during the early booting stage significantly improved yields particularly of KDML105. Spd also increased K^+ and reduced Na^+ concentration in the mature grains, leading to higher K^+/Na^+ ratio (Saleethong et al, 2013).

Over the past several years, proteomics studies in rice have provided a more critical understanding towards functions of rice proteins under favourable as well as various stress conditions. A proteomic approach was adopted to identify proteins that increase in abundance in leaf sheaths, roots, and leaf blades of 2-week-old rice seedlings subjected to salt stress (Abbasi and Komatsu, 2004). More than 1 100 proteins were reproducibly detected, including 34 up-regulated and 20 down-regulated ones when rice seedlings were exposed to NaCl stress (Yan et al, 2005). Kim et al (2005) identified 47 salt-induced proteins which were involved in major metabolic processes, such as photosynthetic assimilation of carbon dioxide, photorespiration and oxidative damage. In rice leaf lamina, Parker et al (2006) found that 32 out of 2 500 proteins were significantly regulated by salinity. Zhang et al (2009) identified an apoplastic protein in rice roots and found that 10 protein spots which were significantly increased or decreased in abundance during the initial phase of salt stress. These proteins included some well-known biotic and abiotic stress related proteins such as *O. sativa* root meander curling which drastically increased in abundance. Lee et al (2011) investigated salt-induced leaf proteins in two rice cultivars with different salt sensitivity and found 23 up-regulated protein spots, of which six were newly reported (class III peroxidase 29 precursor, beta-1,3-glucanase precursor, a putative transcription factor, putative chaperonin 21 precursor, Rubisco activase small isoform precursor, and drought-induced S-like ribonuclease). Comparing leaf proteomes of two rice cultivars differing in salt tolerance, ten different up-regulated proteins and elevated levels of corresponding mRNA transcripts were detected in the salt-tolerant rice cultivar Pokkali, while only three proteins were detected in the salt-sensitive rice cultivar KDML105 (Jankangram et al, 2011). Recently, a proteomic study in early salt stressed roots of SnRK2 transgenic rice revealed 52 early salt-responsive protein spots (Nam et al, 2012). The major up-regulated proteins were enzymes related to energy regulation, amino acid metabolism, methyl glyoxal detoxification, redox regulation and protein turnover.

Although several reports on rice proteomes in response to salinity stress are available, few have included a comparison between salt-tolerant and salt-sensitive cultivars, and none have addressed the effects of exogenous growth regulators on modulation of expression of salt-induced proteins. This study aimed to identify proteins differentially expressed in leaves and grains of two rice cultivars differing in salt tolerance, in response to salt stress and also examine their modulated expression due to pretreatment with Spd. This study is expected to contribute to continuing efforts to determine key processes involved in salt stress tolerance and elucidate the beneficial mechanisms elicited by exogenous Spd.

MATERIALS AND METHODS

Plant materials and salinity treatments at the vegetative stage

Two rice cultivars, Pokkali (salt-tolerant) and KDML105 (salt-sensitive), were used. Seeds were surface-