

学 位 論 文 の 要 旨

論文題目 Molecular Physiological Study on the Adaptive Mechanisms to Salinity Stress in Egyptian Rice Cultivars
(エジプト産イネ品種の塩ストレス適応機構についての分子生理学的研究)

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Soil salinity is one of the most severe problems in agriculture. Absorption of excessive salt inhibits both root and shoot growth, reduces reproductive activity and affects viability of plants. To counter salinity stress, plant cells have several adaptive mechanisms. However, the molecular mechanisms regulating biochemical and physiological changes in response to salinity stress are not well understood. Rice, *Oryza sativa* L., is one of the most important crop species and the major food crop for much of the world's population. Since it is a relatively salt-sensitive crop species, it is important to understand the mode of adaptation of the plant to salinity stress in order to produce new salinity tolerant rice varieties with increased productivity. In Egypt, soil salinization is becoming increasingly challenging for agriculture, and the response of many rice cultivars to salinity stress has not been clearly established. Thus, our study was conducted to investigate the physiological responses of two important local rice cultivars to salinity stress and to elucidate differences in the mechanisms of salinity tolerance between them by comparing the growth parameters, Na⁺ and K⁺ accumulation and the expression profiles of some genes encoding Na⁺ and K⁺ transport protein. Also, to elucidate the further molecular mechanisms of stress tolerance in the salinity-tolerant cultivar, through the isolation and characterization of genes which could be involved in other tolerance pathways that might reveal other adaptation strategies of this cultivar in response to salinity stress.

1. Growth, physiological adaptation and gene expression analysis of two Egyptian rice cultivars under salt stress

To investigate the mode of adaptation under salinity stress, the physiological parameters of two local Egyptian rice cultivars, Sakha 102 and Egyptian Yasmine were examined under 50 mM NaCl stress for 14 days. The results indicated that Egyptian Yasmine was relatively salt tolerant compared Sakha 102, and this was evident in its higher dry mass production, lower leaf Na⁺ levels, and enhanced water conservation under salt stress conditions. Moreover, Egyptian Yasmine exhibited lower Na⁺/K⁺ ratios in all tissues under salinity stress. In contrast, Sakha 102 appeared salt sensitive and accumulated much higher Na⁺ in the leaves, with reduced growth and higher Na⁺/K⁺ ratio especially in the leaves. Therefore, the adaptation of Egyptian Yasmine to salt stress involves reduced Na⁺ accumulation in the leaves, which might be due to a mechanism which excludes Na⁺ from shoot, and that this mechanism is not operating in Sakha 102. However, this mechanism of Na⁺ exclusion needs to be elucidated, in order to understand the basis for the differences in Na⁺ accumulation

between the two cultivars. Na^+/K^+ transport proteins have been shown to control the transport of Na^+ and/or K^+ across membranes and regulate ion homeostasis in cells. We analyzed the transcript levels of some key genes encoding Na^+ and K^+ transport proteins in different tissues of Egyptian Yasmine and Sakha 102. Moreover, in response to salinity stress, Egyptian Yasmine showed induction of expression of some membrane transporter/channel genes that may contribute to Na^+ exclusion from the shoots (*OsHKT1;5*), limiting excess Na^+ entry into the roots (*OsLti6b*), K^+ uptake (*OsAKT1*), and reduced expression of a Na^+ transporter gene (*OsHKT2;1*). Therefore, the active regulation of genes related to Na^+ transport at the transcription level might be involved in salt tolerance mechanisms of Egyptian Yasmine. Differences in the mechanisms of salinity tolerance between the two cultivars may be partly explained by the distinct regulation of gene expression of Na^+ and K^+ transport proteins, and these mechanisms offer the promise of improved salinity stress tolerance in local Egyptian rice genotypes.

2. Identification of a type 3 metallothionein-like gene (*OsMT-3a*) from rice through functional screening analysis in *Escherichia coli*, confers tolerance against salinity and heavy-metal stresses

To further elucidate the molecular mechanism of stress tolerance in Egyptian Yasmine, we set out to isolate and characterize the salinity-inducible genes which could be involved in other adaptation processes under salinity stress conditions. A metallothionein-like type 3 (*OsMT-3a*) gene was identified from rice plants (cv. Egyptian Yasmine) grown under NaCl stress, through cDNA library screening in *Escherichia coli* cells. Heterologous expression of *OsMT-3a* in *E. coli* cells has induced their tolerance to NaCl and heavy metals, Cd^{2+} , Zn^{2+} , and Cu^{2+} (mostly Cd^{2+}) than did control cells, in terms of growth performance. Under high concentrations of H_2O_2 , *OsMT-3a*-overexpressing *E. coli* cells showed enhanced growth, while the growth of control cells was completely inhibited. Hydrogen peroxide levels under NaCl stress conditions in *OsMT-3a*-transformed cells were less than one third those in control cells. Quantitative real-time PCR analysis revealed that expression of *OsMT-3a* was highly induced by salinity stress in the leaves of the salinity-tolerant cultivar Egyptian Yasmine, but not in those of the salinity-sensitive one, Sakha 102. *In vivo* localization of oxygen superoxide (O_2^-) and quantification of H_2O_2 in the leaves of the rice plants grown under NaCl and CdCl_2 stress conditions showed that, Egyptian Yasmine maintained lower levels of O_2^- and H_2O_2 concentrations compared to those in Sakha 102. Taken together, these results suggested that, heterologous expression of *OsMT-3a* has conferred tolerance to NaCl and heavy metals and reduced the deleterious effects of H_2O_2 stress in *E. coli* cells, and that *OsMT-3a* can potentially contribute to the tolerance of rice plants to oxidative stress brought about by salinity and heavy metal stresses, through its ability to scavenge ROS.

In conclusion, the divergent regulation of Na^+ and K^+ transporters may be involved in the maintenance of lower Na^+/K^+ ratios in Egyptian Yasmine under salt stress. Moreover, further evaluation of salinity stress-induced genes in Egyptian Yasmine yielded the *OsMT-3a* gene, whose overexpression in *E. coli* showed that it is important in mediating ROS scavenging. Overall, these studies have shown that the tolerance of Egyptian Yasmine to salinity stress lies in its ability to exclude Na^+ in the leaf and to scavenge ROS.