

学位論文の要旨

論文題目 Molecular Physiological Study of Saline-Alkaline Stress Tolerance in Rice
(イネの塩アルカリストレス耐性に関する分子生理学的研究)

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Soil salinization/alkalinization is an important agricultural contaminant and has complex effects on plant metabolism. The combinations of high Na^+ and high pH stresses in saline-alkaline soils have more severe effects on plant growth and development than high Na^+ stress in saline soils. In order to overcome the toxic effects caused by saline-alkaline stress, plants have to use several adaptive strategies. However, both physiological and molecular mechanisms behind saline-alkaline tolerance in plants have not been fully elucidated. Rice is classified as a glycophyte which is relatively sensitive to salinity stress. Although rice is cultivated in many regions around the world, a significant reduction in yield is frequently observed under saline-alkaline stress environments. In past decades, a few rice genotypes have been analyzed for their combined saline and alkaline responses. Thus, the present study was conducted to investigate the molecular physiological responses of two well-known rice genotypes (FL478; salt-tolerant genotype, and IR29; salt-sensitive rice genotype) to different pH of saline-alkaline stress conditions and to elucidate differences in the mechanisms of saline-alkaline tolerance between them by comparing the several growth parameters; Na^+ and K^+ accumulation, expression profiles of the genes that encode Na^+ and/or K^+ transport proteins together with Fe acquisition proteins. In order to produce new saline-alkaline tolerant rice genotypes in the future, 17 rice genotypes were screened by hydroponic culture to identify tolerant rice genotypes to a wide range of saline-alkaline stress conditions. Then, the physiological mechanisms behind the saline-alkaline tolerance in the selected-rice genotype (Fukoku) were analyzed by comparing their growth parameters with the sensitive rice genotype (IR29).

1. Growth, physiology, and transcriptional analysis between the salt-tolerant and the salt-sensitive rice genotypes under saline-alkaline stress conditions

To investigate the physiological responses under saline-alkaline stress, the growth parameters of two well-known rice genotypes; FL478 (the salt-tolerant rice), and IR29 (the salt-sensitive rice) were examined under saline-alkaline stress conditions at either pH 9 + 50 mM Na (severe), pH 8 + 50 mM Na (moderate), and pH 7 + 50 mM Na (mild) for 3 weeks. The results indicated that FL478 was relatively saline-alkaline tolerant compared to IR29, and this was evident in its higher dry mass production, lower leaf Na^+ concentration in the leaf blades, and enhanced water conservation under both mild and moderate saline-alkaline stress conditions. In addition, under pH 7 and pH 8 of saline-alkaline stress conditions, FL478 plants can maintain lower Na^+/K^+ ratios in the photosynthetic tissues (both leaf blades and leaf sheaths) than IR29 plants. Thus, the greater tolerance of FL478 plants to saline-alkaline stress is related to low Na^+ accumulation in the shoots, which might be due to a mechanism which excludes Na^+ from roots, and that this mechanism may not be operating in IR29 plants. In order to understand the mechanisms underlying differential Na^+ accumulation in two rice genotypes, some important genes encoding Na^+ and K^+ transport proteins together with Fe acquisition proteins were analyzed in the roots. In

response to mild and moderate saline-alkaline stresses, FL478 plants had highly induced expression of some membrane transporter/channel genes that may contribute to low Na⁺ accumulation in the shoots (*OsHKT1;5* and *OsSOS1*) and also induced expression of the genes for K⁺ acquisition (*OsAKT1*, *OsHAK7*, *OsHAK10*, and *OsHAK17*), Fe acquisition (*OsNAS1*, *OsNAS2*, *OsIRT1*, and *OsIRO2*), and rhizosphere acidification (H⁺-ATPase-encoding genes). Therefore, our results highlight that a higher expression of the genes encoding Na⁺ and K⁺ transport proteins together with Fe acquisition proteins may confer greater tolerance of FL478 plants to mild and moderate saline-alkaline stress conditions. Differences in the mechanisms of saline-alkaline tolerance between the two rice genotypes can be clearly explained by the distinct regulation of genes encoding Na⁺ and K⁺ transport proteins together with Fe uptake-related genes.

2. Identification of rice genotypes with high tolerance to long-term saline-alkaline stress

In previous study, 93 rice genotypes (including both *indica* and *japonica* subspecies) were assessed their tolerance to saline-alkaline stress at pH 8.5 + 50 mM Na. Among these rice genotypes screened, 17 rice genotypes were classified as saline-alkaline tolerant. Therefore, this study was conducted to identify the most saline-alkaline tolerant rice genotype by using 17 rice genotypes selected from the previous study. The results obtained from all experimental screenings have been confirmed that Fukoku is relatively saline-alkaline tolerant compared to other rice genotypes. To gain a better understanding of mechanism underlying saline-alkaline tolerance of Fukoku plants, several growth parameters between the most saline-alkaline tolerant Fukoku and the saline-alkaline sensitive IR29 were investigated after 4 weeks of saline-alkaline stress condition at pH 8.5 + 50 mM Na. Fukoku plants displayed better growth performance, as it maintained greater shoot elongation and dry weight and higher concentrations of total chlorophyll, proline, and microelements, while it had a lower Na⁺ concentration in both shoot and root tissues and a lower concentration of malondialdehyde, and a lower Na⁺/K⁺ ratio in the leaf blades, in comparison to IR29 plants. In addition, to test whether saline-alkaline tolerance in rice plants is driven by the effects of high Na⁺ tolerance or high pH tolerance, the growth parameters of two rice genotypes were further examined under saline (50 mM Na), alkaline (pH 8.5 + 5 mM K), and saline-alkaline (pH 8.5 + 50 mM Na) stress conditions. This study found that maintaining a low Na⁺ concentration in the shoots and roots of Fukoku plants is considered as one important strategy to decrease the deleterious effects caused by high Na⁺ stress in both saline (50 mM Na) and saline-alkaline (50 mM Na + pH 8.5) treatments. Besides having a salt tolerance mechanism, Fukoku plants also have a greater ability to tolerate to high pH stress of alkaline treatment (pH 8.5 + 5 mM K), which allow this rice genotype to acquire micronutrients more efficiently under the combinations of high pH and high Na⁺ stresses in saline-alkaline treatment. Differences in the mechanisms of saline-alkaline tolerance between Fukoku and IR29 rice genotypes could be explained by the distinct regulation of genes encoding Na⁺ transport proteins and genes for Fe acquisition proteins. In response to saline-alkaline stress at pH 8.5 + 50 mM Na, Fukoku showed induction of expression of *OsSOS1*, *OsHKT1;5*, and *OsIRO2* genes that may contribute to Na⁺ exclusion

from the roots, restriction of Na⁺ accumulation in the leaves, and Fe homeostasis, respectively. However, IR29 had lower expression of those genes in its roots after exposure to saline-alkaline stress condition (pH 8.5 + 50 mM Na) for 4 weeks. Thus, this study suggests that the rice genotype identified here will provide useful genetic traits to develop new rice genotypes having saline-alkaline tolerance.