学位論文の要旨

論文題目 Molecular Physiological Characterization of Tissue Tolerance in Rice under Salt Stress（塩ストレス下におけるイネの組織耐性の分子生理学的解析）

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Soil salinity is a significant abiotic stress in agricultural crop productivity worldwide. One crop that has suffered a decline in productivity due to high salinity is rice. Rice is a glycophyte known as a sensitive plant to high sodium environments. On the other hand, rice is the most widely consumed food crop, and due to population growth, the global rice demand is increasing. Therefore, improving rice resistance to salinity is essential to meet the yearly increase in rice demand.

There are apparent differences in salinity tolerance mechanisms among rice varieties. Exploration of rice landraces or traditional cultivars with salt tolerance traits can be one solution to overcome the decline in rice productivity due to high salinity. In order to find rice genotypes that have the potential for salt tolerance traits, screening rice genotypes that are tolerant to salt stress is essential. Thus, the present study was conducted to identify rice genotypes from the local Japanese variety that are tolerant to salt stress during the vegetative stage. Furthermore, characterization of the mechanism underlying the salt tolerance in the identified salt tolerance (Shuzenji-kokumai, SZK) and elucidation of its transcriptomic profile, followed by identifying genes associated with tissue tolerance in SZK, was conducted.

Fifteen rice varieties were screened by hydroponic cultivation to identify the tolerant rice genotypes with better growth under salt stress conditions. This experiment identified a Japanese rice variety, SZK, as salt-tolerant with a unique response under salt-stress conditions. This variety can maintain high biomass while having a high sodium concentration in the shoot. These manners differ from FL478, which can exclude sodium from shoots in response to salt

stress, so FL478 shows low sodium concentration in shoots. Based on those findings, this variety is considered to have a tissue tolerance ability.

Analyzing the difference in salt tolerance between the rice genotypes could give a better understanding of the salinity tolerance mechanism in rice so that improving rice resistance to salinity could be achieved. Both physiological and molecular mechanisms behind the salt tolerance in SZK were analyzed by comparing its physiological and molecular characteristics with FL478 and Kunishi, which are salt-tolerant and salt-sensitive, respectively. Under salt stress, SZK accumulates high sodium in the shoot, almost as high as the salt-sensitive Kunishi. The electrolyte leakage ratio and malondialdehyde concentration of SZK do not change significantly under salt stress, indicating that salt stress does not affect the membrane damage of SK. The transcript levels of the genes encoding sodium transporters were analyzed to understand the mechanisms behind the differences in salt tolerance between the three genotypes. In response to salt stress, FL478 showed induction of expression of *OsHKT1;5* genes in roots that may contribute to sodium exclusion from the shoots, while SZK showed induction of expression of *OsNHX2* gene in the leaf sheaths. This result implied that SZK could compartmentalize Na^+ in the vacuole through the function of *OsNHX2* to avoid sodium toxicity in the cytosol and maintain better physiological status under salt stress. RNA-seq analysis was then performed to find transcriptomic profiles of SK under salt stress. Among the 4623 and 1998 DEGs in the leaf blade and leaf sheath, respectively.

The expression of *OsHSP90*, *OsHsp20, OsHsp24.1,* and *OsHsfA2a* were found to be up-regulated specifically in the leaf sheaths of SZK, indicating that these genes may associated with tissue tolerance in SZK. HSPs function as a general chaperone that transiently binds to folding intermediates in vitro, prevents aggregation and supports refolding intermediates to their native states (Liu et al. 2009; Raman and Suguna 2015; Schopf et al. 2017). Protein synthesis requires high concentrations of K^+ for the binding of tRNA to ribosomes (Blaha et al. 2000; Tester and Davenport 2003) and to preserve ribosome integrity (Rozov et al. 2019). Under salt stress conditions, protein synthesis was disrupted because K^+ concentration in the cell is lower and cannot be substituted by Na^+. Proteins that are formed incorrectly will be degraded by the proteasome or fixed by molecular chaperone. The ability of SZK to maintain a better physiological status than the salt-sensitive variety while having a high Na^+ concentration may occur under the activity of HSPs, which prevents protein damage, allowing the plants to retain active cellular metabolisms and growth.

Functional analysis of several novel genes using yeast complementary assay showed that *OsCYP76C2, OsLAC28,* and *OsLOX-L2* genes may play a role in SZK tissue tolerance. However, no studies have reported the function of these genes in salt tolerance in rice. Although there is no information regarding the roles of *OsCYP76C2, OsLAC28,* and *OsLOX-L2* in salt tolerance, our results suggest that these three genes may confer tissue tolerance to SZK. Further investigation of these three genes is required to understand their roles in salt tolerance, particularly tissue tolerance.