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主　論　文　の　要　旨

論文題目　Physiological and molecular mechanisms of salt removal by leaf sheath in rice
（イネの葉鞘における塩排除の生理・分子機構）

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論　文　内　容　の　要　旨

Soil salinization significantly limits rice production across the globe due to the fact that rice is hypersensitive to salt stress with a low threshold electrical conductivity of 3 dS m^{-1} equivalent to 30 mM NaCl. Salt sensitivity in rice plants is associated with the accumulation of Na^+ and Cl^- to the toxic level in shoots and particularly in the leaf blade which is the most active photosynthetic tissue. Therefore, the removal of Na^+ and Cl^- from the leaf blade is important for the survival and salt tolerance in rice. To remove Na^+ and Cl^- toxic ions from the leaf blade in rice, these ions are unloaded from xylem vessels and sequestered to the vacuole in the leaf sheath of rice, which referred to as 'salt removal ability in leaf sheath'. The general objective of this study was to investigate physiological and molecular mechanisms of salt removal by the leaf sheath in rice to determine the control point of and genes associated with the ability of removing salt in the leaf sheath of rice.

In Chapter 2, the physiological mechanism of salt removal in the rice leaf sheath was characterized by studying on specific part and tissue involved in the accumulation of salt ions including Na^+, Cl^- and K^+ in the leaf sheath. To achieve this goal, the distribution patterns of Na^+, Cl^- and K^+ was identified along the longitudinal axis of leaves and in the internal tissues of leaf sheath of salt-tolerant variety FL478 and salt-sensitive variety IR29 grown under control and saline conditions. The measurement and specific locations of Na^+, Cl^- and K^+ were examined using atomic adsorption spectrometry, ion chromatography and energy dispersive X-ray spectroscopy attached to a scanning electron microscope. The removal ability of Na^+ and Cl^- was evaluated by the sheath:blade ratio of Na^+ and

Cl^- concentrations. The results illustrated that the leaf sheath of rice efficiently functions to remove excess Na^+ and Cl^- from xylem vessels in the basal part (Na^+) and in the upper part (Cl^-) along the longitudinal axis. After transferring Na^+ and Cl^- from xylem vessels, these ions were transported to and sequestered in the central fundamental parenchyma cells of leaf sheaths under salt stress.

In Chapter 3, gene expression analysis was performed to identify Na^+, Cl^- and K^+ transporter genes associated with the salt distribution patterns along the longitudinal axis of leaves and in the internal tissues of leaf sheaths, and the genes that increase their expression levels under salinity. The 5th leaves of salt-tolerant variety FL478 treated with NaCl was used and separated into 4 parts: basal, middle, and apical parts of leaf sheath and whole leaf blade. Real-time PCR analysis was performed to determine the relative expression levels of each candidate gene. The transcriptional expression analysis indicated that *OsHKT1;1*, *OsHKT1;5*, *OsNHX1*, *OsNHX2*, *OsNHX3* and *OsNHX5* might be involved in the accumulation of Na^+ in the basal parts of salt-treated leaf sheath. *OsHKT1;5* may be involved in Na^+ unloading from xylem vessels. The accumulation of Na^+ in the fundamental parenchyma cells might be mediated by *OsNHX3* in the central regions and *OsNHX5* in the peripheral regions under saline conditions. Furthermore, the removal of Cl^- in the leaf sheath is possibly regulated by *OsNPF2;4*, *OsCLC1*, *OsCLC2*, *OsSLAH1* and *OsSLAH2*. The Cl^- accumulation in the fundamental parenchyma cells might be associated with *OsNPF2;4* and *OsCLC2* in response to salt stress.

In Chapter 4, genome-wide association study (GWAS) was conducted to find associated single nucleotide polymorphisms (SNPs) and candidate genes related to the salt removal ability in the rice leaf sheath. In this study, 296 accessions of the rice diversity panel, composed of *indica*, *aus*, *tropical japonica*, *temperate japonica*, *aromatic* and *admixed* accessions, were used to identify salt removal related traits. Genome association analysis was performed using a 44 K SNP chip. The GWAS results showed that 11 SNPs were significantly associated with the removal of Na^+ in leaf sheath from the *indica* subpopulation and that numerous SNPs were significantly associated with the removal of Cl^- in the leaf sheath from whole panel as well as from *indica* and *tropical japonica* subpopulations. 25 genes and 211 genes were discovered in the significantly associated regions with Na^+ and Cl^- removal ability in the leaf sheath, which derived from many functional categories such as transport, enzymes, kinase activity, protein binding and many others. GWAS data implied that rice accessions in the *indica* sub-group are the main source of genes or alleles that regulate Na^+ and Cl^- removal in leaf sheaths, while the removal of Cl^- in leaf sheaths is probably involved in multiple genetic mechanisms under salt stress.

Taken all together from Chapters 2 to 4, the leaf sheath of rice actively removes Na^+ in the basal parts and Cl^- in the upper parts by unloading these ions from xylem vessels and transferring them to the central regions of internal leaf sheath. Then, Na^+ and Cl^- were highly accumulated in the fundamental parenchyma cells in the central regions of internal leaf sheath, which in turn helps to reduce the concentrations of these ions in the leaf blade. The removal of Na^+ in the leaf sheath may be genetically associated with six Na^+ transporter genes and some of 25 genes detected in GWAS on Na^+ sheath:blade ratio. Five Cl^- transporter genes and some of 211 genes found in GWAS on Cl^- sheath:blade ratio might be involved in the Cl^- removal ability in the rice leaf sheath. Additionally, *indca* rice varieties are the important sources of diverse genes that regulate the Na^+ and Cl^- removal ability in the leaf sheath of rice. The outcomes of this project will help enhance the understand of complex salt tolerance mechanism in rice and provide potential candidate genes for future molecular studies and breeding program, which is crucial to create superior salt-tolerant rice varieties.