698(F813)

TWO TYPES OF HKT HOMOLOGUES WITH DIFFERENT PROPERTIES OF Na^+ AND K^+ TRANSPORTATION IN *Oryza sativa*

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To better understand the Na^+ and K^+ homeostasis in important crop rice (*Oryza sativa* L.), a cDNA homologous to the wheat *HKT1* was isolated from japonica rice, cv Nipponbare (Ni-*OsHKT1*) and also from salt-tolerant indica rice, cv Pokkali (Po-*OsHKT1*, Po-*OsHKT2*). We characterized the ion-transport properties of OsHKT1 and OsHKT2 using an expression system in the heterologous cells, *Saccharomyces cerevisiae* and *Xenopus laevis* oocytes. *OsHKT2* was capable of completely rescuing a K^+-uptake deficiency mutation in yeast, whereas OsHKT1 was not under K^+-limiting conditions. When OsHKTs were expressed in Na^+-sensitive yeast, *OsHKT1* rendered the cells more Na^+-sensitive than did *OsHKT2* in high NaCl conditions. Finally, the presence of K^+ caused positive shifts in the reversal potentials of *OsHKT2*-expressing oocytes but had little effect on oocytes expressing *OsHKT1*. The results demonstrated that OsHKT1 is an AtHKT1-type transporter, while OsHKT2 is a wheat HKT1-type transporter.

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THE FUNCTIONAL ANALYSIS OF THE RICE Na^+/H^+ ANTIPORTER GENE

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Vacuolar Na^+/H^+ antiporter transports Na^+ from the cytoplasm to vacuoles using a pH gradient generated by proton pumps. Overexpression of a vacuolar Na^+/H^+ antiporter gene, *OsNHX1*, which we isolated from rice, suppresses the salt and hygromycin sensitivity of the yeast *nhx1* mutant. The expression of the gene is increased by salt stress. These results suggest that the antiporter is responsible for salt-tolerance in rice. In addition, the OsNHX1 protein was detected mainly in the rice tonoplast-enriched fraction using the anti-OsNHX1 antibody. Based on these results, transgenic rice overexpressing the gene was produced, and the functional analysis was carried out. In the experiment using the transgenic rice callus, the callus overexpressing the gene shows the higher salt tolerance than the wild type. We are analyzing the phenotype of the transgenic plant.

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INTERCELLULAR LOCATION AND ESTIMATION OF CHLORIDE CHANNELS FROM RICE

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During conditions of high salt concentrations, plant cells translocate ions to vacuoles, as well as to the extracellular environment to reduce ionic stress in the cytoplasm, to maintain a high osmotic pressure in the vacuole. A Na^+/H^+ antiporter transports Na^+ removal or accumulation from/to cells. Cl^- ion is a major anion, which accumulate under the salt stress and have an important role in cellular homeostasis.

We isolated two kinds of Cl channel genes (OsCLC-1 and -2) from rice and examined the intercellular localization of the protein product of both clones. The results of western blot analysis indicates that the OsCLC-2 protein is mainly localized in the tonoplast fraction. GEF1, which is a single CLC gene in *Saccharomyces cerevisiae*, is necessary for the maintenance of the ionic environment in yeast, localizes in the Golgi compartment or vacuoles. Yeast complementation tests were performed to OsCLC-1 and -2 in a GEF1 disruptant mutant. Here, we propose a function for OsCLC-1 and -2 based on results of GFP-localization analysis and the results of a yeast complementation assay.

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ISOLATION AND CHARACTERIZATION OF THE *BOR1* GENE

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Boron (B) is an essential element of higher plants. It has been demonstrated that one of the physiological functions of B is to maintain the structure of the cell wall. On the other hand, mechanisms of uptake and transport of B are still not clear. In general, it has been considered that uptake and transport of B are passive processes.

An *Arabidopsis thaliana* mutant, *bor1-1*, is sensitive to B deficiency. Our study demonstrated that *A.thaliana* plants have a B concentration mechanism in root-to-shoot translocation only at low B supply, and the major defect in the *bor1-1* plants is in this mechanism. Therefore, it is considered that the physiological function of *BOR1* gene is in the B concentration mechanism.

Recently, we identified the *BOR1* gene. Here we report the process of cloning and analysis of structure and expression pattern of the *BOR1* gene.