論 文 の 内 容 の 要 旨

生産・環境生物学専攻
平成１９年度博士課程 入学
氏名　シントー　ワユニング　アルディエ
Sintho Wahyuning Ardie
指導教員名　高野　哲夫

論文題目　Studies on potassium and sodium transport genes in rice and *Puccinellia tenuiflora* in response to salt stress

（イネおよび *Puccinellia tenuiflora* における、塩ストレス下のカリウムおよびナトリウム輸送体遺伝子に関する研究）

Increased salinization of arable land is greatly reducing yield much below the genetic potential of the cultivated plants. Consequently, new strategies including both genetic manipulation and traditional breeding approaches represent a major research priority to enhance crop yield stability on saline soils. Therefore, an understanding of physiological and genetic mechanisms how plants tolerate and acclimate to saline environments is of great importance for genetic modification and plant breeding. Since the capacity of plants to maintain a high cytosolic K^+/Na^+ ratio is one of the key determinants of plant salt tolerance, identification of K^+ and Na^+ transport systems would lead to a better understanding about salinity tolerance mechanisms. Plants differ greatly in their tolerance of salinity, as reflected in their different growth responses. Most crops are glycophytes, thus are not capable of growing under high saline conditions. Halophytes, plants adapted to saline habitats, have evolved various mechanisms to overcome salt stress in the long-term natural selection. Therefore, understanding the salt-tolerance mechanism(s) of monocotyledonous halophytes will aid in improving the salt tolerance of cereals. This study focused on the isolation and characterization of K^+ and Na^+ transport genes from a halophytic plant, *Puccinellia tenuiflora* (*P. tenuiflora*).

(1) Cloning of a high-affinity K^+ transporter gene *PutHKT2;1* from *P. tenuiflora* and its functional comparison with *OsHKT2;1* from rice in yeast and *Arabidopsis*

HKT-type transporters have been characterized in several plant species and the known plant HKTs have been shown to perform diverse functions. TaHKT2;1 functions as a Na^+-uptake pathway in wheat roots, while rice OsHKT2;1 mediates Na^+-uptake into K^+-starved roots. In *Arabidopsis*, AtHKT1;1 functions in retrieval of Na^+ from the transpiration stream. These previous studies showed the important role of HKT transporters in plant salt tolerance.

In this study, a high-affinity K^+ transporter *PutHKT2;1* cDNA from the salt-tolerant plant *P. tenuiflora* was isolated. *PutHKT2;1* belongs to subfamily 2 in HKT-family by a phylogenetic analysis. By using the green fluorescent protein (GFP), the PutHKT2;1 protein was shown to localized in the plasma membrane. Expression of *PutHKT2;1* was induced by both 300 mM NaCl and K^+-starvation stress in roots, but only slightly regulated by those stresses in shoots. *PutHKT2;1* transcript levels in 300 mM NaCl were doubled by depletion of potassium. Yeast transformed with *PutHKT2;1*, like those transformed with *PhaHKT2;1* from salt-tolerant reed plants (*Phragmites australis*), (i) were able to take up K^+ in low K^+ concentration medium or in the presence of NaCl, and (ii) were permeable to Na^+. This suggests that *PutHKT2;1* has a high affinity K^+-Na^+ symport function in yeast. *Arabidopsis* over-expressing *PutHKT2;1* showed increased sensitivities to Na^+, K^+, and Li^+, while *Arabidopsis* over-expressing *OsHKT2;1* from rice showed increased sensitivity only to Na^+. In contrast to *OsHKT2;1*, which functions in Na^+-uptake at low external K^+ concentrations, *PutHKT2;1* functions in Na^+-uptake at higher external K^+ concentrations. These results show that the modes of action of *PutHKT2;1* in transgenic yeast and *Arabidopsis* differs from the mode of action of the closely related *OsHKT2;1* transporter.

(2) Cloning of an AKT1-type K^+-channel α subunit gene *PutAKT1* from *P. tenuiflora* and its functional analysis in *Arabidopsis*

Plant voltage-dependent K^+ channels have been found to play an important role in K^+ homeostasis in higher plants. Channel-mediated K^+ uptake at the soil-root interface in all plants studied to date has been associated with homologues of the *Arabidopsis thaliana* AKT1 (*Arabidopsis* K^+ transporter 1) channel. Previous studies demonstrated the adverse effect of Na^+ on the K^+ uptake through AKT1-type channel, indicating that the regulation of AKT1-type channel is one important determinant to salt stress tolerance.

In this study, cDNA for α subunit of an inward-rectifying K^+ channel was isolated from the salt tolerant *P. tenuiflora* and designated as *PutAKT1*. The phylogenetic analysis showed that PutAKT1 belongs to AKT1-subfamily in *Shaker* K^+ channel family. *PutAKT1* was localized in the plasma membrane and it was preferentially expressed in the roots.

The expression of *PutAKT1* was induced by K^+-starvation stress in roots and was not down-regulated by the presence of excess Na^+. *Arabidopsis* plants over-expressing *PutAKT1* showed enhanced salt tolerance compared to wild-type (WT) plants. Under 75 mM NaCl stress, the *PutAKT1*-expressing plants showed better shoot phenotype and higher fresh- and dry-weight than that of the WT. Furthermore, by the expression of *PutAKT1*, the K^+ content of *Arabidopsis* increased under normal, K^+-starvation, and NaCl-stress condition. *Arabidopsis* expressing *PutAKT1* also showed a decrease in Na^+ accumulation both in shoot and root. These results suggest that (i) PutAKT1 is involved in mediating K^+ uptake in both low- and high-affinity uptake range, and (ii) unlike its homologues in rice, it seems to mediate K^+ uptake even under salt stress condition.

(3) Functional comparison of K^+ channel α and β subunit of rice and *P. tenuiflora*: The role of K^+ channel α and β subunit interaction in K^+-nutrition

Plant voltage-dependent K^+ channels are multimeric proteins which are composed of homotetrameric α subunit and an auxiliary β subunit. The β-subunits are not required for the K^+ channel to be active. However, they can confer different properties to the channel. Some β-subunits have been shown to change the inactivation rate of their target channels whereas other behave as chaperones promoting channel maturation and increased the stability of K^+ channel protein on the plasma membrane.

We cloned a cDNA for K^+ channel β subunits from the *P. tenuiflora* and named it *KPutB1*. *KPutB1* was preferentially expressed in the roots of *P. tenuiflora*. Potassium starvation, 300 mM NaCl stress, or the combination of both stresses lead to a transient induction of *KPutB1* transcript levels in both roots and shoots. For further analysis, the K^+ channel α and β subunit homologues from rice (*OsAKT1* and *KOB1*, respectively) were also isolated. By yeast two-hybrid assay we demonstrated that KPutB1 interacts with PutAKT1. We also found that the interaction between K^+ channel α- and β-subunits could occur across plant species, as KPutB1 could also interact with rice OsAKT1 and *Arabidopsis* AKT1. In order to understand the functional relevancies of this interaction on K^+-nutrition, co-expression experiments in yeast were conducted under various ionic conditions. Our result showed that yeast co-expressing *PutAKT1* and the β subunits (*KPutB1* and *KOB1*) had a better growth and higher K^+-uptake ability than yeast expressing *PutAKT1* alone. In contrast, co-expressing the β subunits (KPutB1 and KOB1) with *OsAKT1* leads to a reduction in the yeast growth and K^+ uptake rate in comparison to that of yeast expressing *OsAKT1* alone. These results suggest that (i) co-expressing K-channel α and β subunit in yeast would lead to a different growth and different K^+-uptake ability than yeast expressing K-channel α subunit alone, and that (ii) the growth phenotype of the co-expressing yeasts was dependent on the α subunit component. *Arabidopsis* plants over-

expressing K^+-channel β subunit of *P. tenuiflora* or rice showed increased shoots K^+ content and decreased roots Na^+ content under control, 75 mM NaCl, and K^+ starvation stress conditions. However, the different ion accumulation between WT and K^+-channel β subunit-expressing plants does not lead to any phenotypic difference with the WT under salt stress and K^+ starvation conditions.

It was already shown that the salinity tolerance of *P. tenuiflora* depends on its ability in maintaining K^+ uptake and limiting the unidirectional Na^+ influx under salt stress. A low-affinity K^+ uptake system that selectively transports K^+ in *P. tenuiflora* roots have been reported to play important role in the salinity tolerance of this plant, and *PutAKT1* is the candidate of this low-affinity K^+ uptake system. KPutB1 may increase the activity of PutAKT1 and help the K^+ channel to function properly even under unfavorable condition such as salinity. The shoot Na^+ content of *P. tenuiflora* was reported to remain relatively unchanged under salt stress, and our results suggest that PutHKT2;1 may involved in maintaining Na^+ homeostasis of this plants (e.g. by retrieving Na^+ from the transpiration stream). The mechanism of salinity tolerance is a very complex phenomenon and it involves multiple stress responsive genes. Detailed analysis of each gene and also the cross talk within components of stress signal transduction pathway are of great importance. Careful utilization of specific genes, including targeting to specific cell types or tissues, should help in developing salt tolerant cultivar.