論文の内容の要旨

生産・環境生物学　専攻
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氏　　名　張　欣欣
指導教員名　高野　哲夫

論文題目　Functional analysis of the genes in Arabidopsis, rice and *Chrolis virgata* in response to several abiotic stresses.
（環境ストレスに応答する、シロイヌナズナ、イネ、*Chrolis virgata* 遺伝子の機能解析）

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Bray *et al*., 2000; Wang *et al*., 2003; Mahajan and Tuteja, 2005). Therefore, breeding for stresses tolerance in plants should be given high research priority in plant biotechnology programs. Molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress-related genes. In our study, we isolated and characterized several stresses-related genes from *Arabidopsis thaliana*, rice (*Oryza sativa* L.) and wild salt-tolerant plant *Chloris virgata*, to know how these genes respond to abiotic stress and how they conferred on stresses tolerance in yeast cells and plants. The mechanisms of how they involved in the plant stresses tolerance also have been discussed.

(1) Two cysteine proteinase inhibitors (cystatins) from *Arabidopsis thaliana*, designated AtCYSa and AtCYSb, were characterized. Recombinant GST-AtCYSa and GST-AtCYSb were expressed in *Escherichia coli* and purified. They inhibit the catalytic activity of papain, which is generally taken as evidence for cysteine proteinase inhibitor function. Northern blot analyses showed that the expressions of *AtCYSa* and *AtCYSb* gene in Arabidopsis cells and seedlings were

strongly induced by multiple abiotic stresses from high salt, drought, oxidant, and cold. Interestingly, the promoter region of *AtCYSa* gene contains a dehydration-responsive element (DRE) and an abscisic acid (ABA)-responsive element (ABRE), which identifies it as a DREB1A and AREB target gene. Under normal conditions, *AtCYSa* was expressed in *35S: DREB1*A and *35S: AREB1* plants at a higher level than in WT plants, while *AtCYSa* gene was expressed in *35S: DREB2A* plants at the same level as in WT plants. Under stress conditions (salt, drought and cold), *AtCYSa* was expressed more in all three transgenic plants than in WT plants. Over-expression of *AtCYSa* and *AtCYSb* in transgenic yeast and Arabidopsis plants increased the resistance to high salt, drought, oxidative, and cold stresses. Taken together, these data raise the possibility of using *AtCYSa* and *AtCYSb* to genetically improve environmental stresses tolerance in plants.

(2) A cDNA library was prepared from rice (*Oryza sativa* L.) roots grown in the presence of $NaHCO_3$ stress. A cDNA clone isolated from this library was identified by a homology search as a mitochondrial ATP synthase 6 kDa subunit gene (*RMtATP6*; GenBank accession no. AB055076). In transformed yeast and tobacco protoplasts, the RMtATP6 protein was localized in mitochondria using the green fluorescent protein (GFP) marker. Analysis of *RMtATP6* mRNA levels suggested that the expression of this gene was induced by stress from sodium carbonates and other sodium salts. Transgenic tobacco over-expressing the *RMtATP6* gene had greater tolerance to salt stress at the seedling stage than untransformed tobacco. Among the other genes for F_1F_0-ATPase of rice, some were found to be up-regulated by some environmental stresses and some were not. These data suggest that the RMtATP6 protein acts as a subunit of ATP synthase, and is expressed in response to stress from several salts, with the other genes coding for the subunits of the same ATP-synthase. In Arabidopsis, a 6 kDa protein (At3g46430) has been previously purified from *Arabidopsis thaliana* mitochondrial F_1F_0-ATPase. The gene (*AtMtATP6*; GenBank accession no. AK117680) encoding this protein was isolated from *Arabidopsis* and characterized. Northern blot analyses showed that the expression of *AtMtATP6* gene in *Arabidopsis* suspension-cultured cells was induced by several abiotic stresses from salts, drought and cold. Over-expression of *AtMtATP6* gene in transgenic yeast and *Arabidopsis* plants increased the resistance to salts, drought, oxidative and cold stresses.

(3) A plasma membrane H^+-ATPase (PMA) gene (*ChvPMA*) was isolated from a wild salt-tolerant plant *Chloris virgata*. The expression of *ChvPMA* gene in leaves and roots of *Chloris virgata* seedlings under salt stress (NaCl, $NaHCO_3$) was examined. The results showed the *ChvPMA* gene expression was induced by salt stress. The *ChvPMA* gene was fused to the N-terminus of *GFP* gene, and transferred into onion epidermal cells for analyses of intracellular localization. The result showed that the ChvPMA protein was found to be in the plasma membrane of ion epiderm. Because the H^+-ATPase activity is regulated by a C-terminal auto-inhibitory domain that can be displaced by phosphorylation, we analyzed transgenic yeast expressing either wild-type PMA (*ChvPMA*) or truncated *ChvPMA* lacking the C-terminal auto-inhibitory domain (*ChvPMAΔC*) under high salt and pH conditions. The results showed that over-expression of *ChvPMA* and *ChvPMAΔC* in transgenic yeasts increased the resistance to salt and lower pH conditions, especially, the yeast over-expressing *ChvPMAΔC* showed better growth than *ChvPMA* at an external pH 4.0 in the presence NaCl. Transgenic Arabidopsis over-expressing *ChvPMAΔC* also showed the better root growth than that of *ChvPMA* at an external pH 4.0 in the presence NaCl.

The plant responding to abiotic stress involves many genes and biochemical-molecular mechanisms; therefore, the detailed analysis of each gene how they involved in plant tolerance will be very necessary; however, more careful utilization of specific genes, including targeting to different types of cells and organelles, should result in even greater salt-stress tolerance under true field conditions.