

SUMMARY OF DOCTORAL THESIS

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Title: Functions of Unique Stress-Induced Genes from Rice and Its Mechanism of Salinity Tolerance

(イネの新規ストレス誘導性遺伝子の機能解析と耐塩性機構)

In order to elucidate the functional role of two submergence-induced genes *OsARP* (*Oryza sativa* Antiporter Regulating Protein) and *OsMGD* (*Oryza sativa* monogalactosyldiacylglycerol synthase) isolated from rice cultivar FR13A; transgenic tobacco plants were generated and subsequently morpho-physiological and biochemical analyses were conducted in overexpressed tobacco plants under salt and drought stresses. The genes were prepared, cloned and sub-cloned according to standard procedures. Following this, transgenic tobacco plants were generated by *Agrobacterium* mediated transformation system which resulting the generations of several transgenic lines from each gene. Integration of these foreign genes in tobacco genome was confirmed by polymerase chain reaction (PCR) and the gene expression was confirmed by Western-blot analysis.

The *OsARP* gene expression was reported in rice cultivar FR13A under submergence, salt and drought stresses. In public databases, this protein was annotated as putative Os02g0465900 protein of rice. The OsARP protein (molecular mass 25kDa) had the homology with ChaC, which was the regulator of ChaA $\text{Ca}^{2+}/\text{H}^{+}$ cation transport protein in *E. coli*. The *OsARP* transgenic tobacco plants had better growth and vigor than that of wild type plants under salt stress *in vitro* and had showed higher tolerance to salt and drought stresses as detected by higher net photosynthesis and effective PSII photon yield. The OsARP protein was localized in vacuolar membrane (tonoplast) of rice plants through immunogold electron microscopy. We measured the Na^{+} , K^{+} , Ca^{2+} and Mg^{2+} and water contents in the leaves transgenic of tobacco and wild type plants under salt stress. The total ion and water contents of *OsARP* transgenic plants were higher than those of wild type. Transgenic lines retained more Na^{+} in their leaf tissue than wild type plants. It is likely that the toxic effect of Na^{+} in cytosol might be reduced by sequestration into vacuoles through the operation vacuolar antiporter regulator. Tonoplast vesicles isolated from *OsARP* transgenic plants showed $\text{Na}^{+}/\text{H}^{+}$ exchange rates three-fold higher than wild type plants suggesting OsARP on the tonoplasts plays important role in compartmentation of Na^{+} into vacuoles.

The putative rice MGDG synthase (EC 2.4.1.46) gene *OsMGD* was reported to be expressed under submergence, salt and drought stresses in rice cultivar FR13A. The OsMGD protein (molecular mass 39.9kDa) had high homology to the MGDG synthase of *Cucumis sativus*, *Spinacia oleracea* and *Arabidopsis thaliana*. It was found that the

OsMGD transgenic tobacco plants showed better tolerance to salt and drought stresses keeping higher photosynthesis and effective PSII photon yield when compared with wild type. To evaluate the measurable effects of salt stress on lipid composition, the amount of galactolipids and lipid-P, an indicator of total phospholipid mass, was determined. Initially, without salt stress, *OsMGD* transgenic plants had significantly higher levels of MGDG, DGDG and lipid-P than the wild type. Despite large increases in the amount of MGDG and DGDG in *OsMGD* transgenic plants, their fatty acid profiles were almost identical to those of the wild type tobacco plants. The results suggested that the expression of *OsMGD* gene had no effect on the selection of diacylglycerol precursor for MGDG synthesis and subsequent desaturation steps. Upon salt stress, the level of α -linolenic acid (18:3) gradually decreased in MGDG and DGDG in the wild type tobacco plants, and also in the transgenic plants but to a much lesser extent. The overall fatty acid composition of MGDG and DGDG in *OsMGD* transgenic and wild type tobacco plants was unchanged during salt stress, except for slight decreases in levels of α -linolenate only in wild type plants. This indicated that *OsMGD* transgenic tobacco plants maintained greater stability of galactolipid composition, membrane structure and functions in order to cope with salt stress.

Selection of rice cultivars with a higher level of tolerance to salt stresses has attained much importance in rice breeding programs. Of the different breeding options, induced mutations could be utilized for creation of genetic variability, for the selection of mutant varieties with improved agronomic traits. In order to select salt tolerant mutant lines, we screened 400 M₂ families derived from the US tropical japonica rice cultivar 'Drew' after Gamma irradiation. The preliminary screening was conducted in salt culture and on the basis of seedlings survival showed that 22 M₂ families possessed tolerance to 10dSm⁻¹ (~100mM NaCl) of salt stress. In the next experiment, four-week old seedling of these 22 M₂ families and the parent were exposed to NaCl stress (10dSm⁻¹) throughout the rest of the growing period. The stress imposition resulted in plants showing reduction in growth, leaf rolling and drying, formation of fewer numbers of tillers, delayed flowering and ultimately high to severe grains sterility in most of the families as well as in the parent. Finally, 4 mutant lines out of 22 M₂ families were selected as salt tolerant on the basis of their agronomic performances. It was found that the selected mutant lines gave 8 to 9 times as much grain yield as the parent under salt stress and this yield was 45-60% of the grain yield of the parent without salt stress. Anti-oxidant enzymes activity was determined in the salt tolerant lines as well as their parent under 200mM NaCl stress. Enhanced activities of the ascorbate peroxidase (APX), glutathione reductase (GR), and superoxide dismutase (SOD) were found in the selected mutants under salt stress. This indicated that mutant lines got the ability to up-regulate their antioxidative system in order to cope with the salt stress. Therefore, it was concluded that increased antioxidant enzyme activity was one of the NaCl-stress tolerance mechanisms of the salt tolerant rice mutants was found in this study.