Molecular Genetical Analysis of Mechanisms of Salt Tolerance in Plants

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Summary

Accumulation of salts derived from sandy soil on the land surface by desiccation is a serious problem even when irrigation is partially possible, although the irrigation is needed to make dry desert areas green. Irrigation with diluted sea water may be available in areas closed to sea. In any cases, it is desired to confer the salt tolerance ability on plants. Therefore, we intended to characterize genes regulating to make plants salt tolerant and further bestow that ability on cultivating plants. We have applied genetical methodology to the model plant *Arabidopsis thaliana*, which is suited for mutagenesis and the subsequent gene cloning by complementation.

It is speculated that the rate of formation of the photosynthetic machinery is reduced under salt stress conditions, resulting from suppression of constitutive expression of genes for photosynthesis. We have focused on well known genes for photosynthesis, the genes for the small subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (*RBCS*) and for chlorophyll *a/b*-binding proteins (*CAB*, *LHC*), especially their gene members *RBCS-3B* and *CAB1* (*LHCB1*At3*) of *A. thaliana*. In order to monitor their expression, several reporter genes were placed under the controls of their promoter regions including their upstream sequences.

A. thaliana grown in one week after germination on a standard medium has become bleached during one to two weeks after transferring to media containing 200 to 250 mM NaCl (approx. a half of NaCl concentration in sea water). This sensitivity of *A. thaliana* to salt was variable in each experiment, speculated to be caused by difference in light intensity illuminating plants. The survival rate of *A. thaliana* maintained on a salt medium for four to five days under light less than 1,000 lux (13 μmol m^{-2} sec^{-1}) was 90% or more, and that under 3,000 lux decreased to 10 to 30%. Expression of *CAB1* monitored by luciferase activity in surviving plants showed maximal at illumination of approx. 1,000 lux.

Germination of *A. thaliana* ecotype Columbia and Bensheim was less sensitive to 200 mM NaCl (10 to 20% in germination rate of that on the standard medium) and ecotype Norway and WS was quite sensitive (no germination). The resistance of plant growth to 200 mM NaCl as indicated by survival rate of plants grown for one week after germination during the subsequent two weeks, was high in case of ecotype Bensheim, and that as judged by formation of true leaves was high with ecotype WS. To analyze genes responsible for salt tolerance, genetical crossing of those resistant ecotypes and sensitive ones is being achieved.

The 12,400 lines mutagenized with ethylmethane sulfonate (EMS) and 4,900 lines of insertion mutagenesis with T-DNA were exposed to 200 to 250 mM NaCl after germination. A few lines of salt-tolerant mutants designated "ste" (salt-tolerant expression of photosynthesis genes) were obtained and are further being genetically analyzed.

Salt tolerance of *A. thaliana* is concluded to depend on light intensity and genetically controlled by quantitative trait loci (QTL) which are not simply explained by Mendel's laws.