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Analysis of Genes for Salt Tolerance by DNA Array in *Arabidopsis*

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Summary

Salt tolerance in plants has been attributed to many mechanisms, including accumulation of osmoregulatory solutes inside cells, enhanced ability of cells to take up water or K^+ from surrounding solutions of higher salt concentration, elimination of excess NaCl, and the ability to maintain photosynthetic activity under salt stress. To further investigate the mechanisms of salt tolerance, we have begun to screen and characterize mutants of *Arabidopsis thaliana* tolerant to high NaCl concentrations during photoautotrophic growth. Several mutants of *Arabidopsis* with altered responses to salt have been reported, including mutants tolerant of salt during germination and mutants that are salt-hypersensitive. However, there were no reports of mutants whose photoautotrophic growth was salt tolerant. We have already selected several *pst* (*photoautotrohic salt tolerance*) mutant lines.

Higher expression of gene for putative bHLH transcription factor (bHLH19; At2g22760; bHLH, basic helix-loop-helix, a DNA-binding protein motif) was first detected specifically in *pst2* mutant plants by microarray of approx 15,000 genes, and confirmed by real-time RT-PCR. The abundant species of this mRNA present in plants grown without salt stress was “completely-spliced”, whereas the major species generated under salt stress was “incompletely-spliced”. The intracellular localization of bHLH19 molecules encoded in either completely or incompletely-spliced mRNA was determined by transient expression of the proteins fused to sGFP, resulting in restrictive presence in the nucleus as we expected to function as a transcription factor. The function of bHLH19 was examined with plants transgenic with constructs to express these mRNA species. The wild-type *Arabidopsis* (Columbia) and knocked-out one were sensitive to 75 mM NaCl in the MS solid medium, although lines transgenic with completely- or incompletely-spliced species of cDNA were tolerant to the salt stress during germination and its subsequent growth. The salt-induced and alternatively-spliced species tended to be highly tolerant. The autotrophic growth of plants transformed with bHLH19 cDNA fragments after germination was also tolerant to salt. Furthermore, the transgenic plants exhibited the high tolerance to cold temperature at 4^oC for 4 weeks. The bHLH19 may be the most crucial for the novel networks of signal transduction for acclimation and tolerance to salt and cold temperature.