P 59 Genetic Screening for Salt Stress Related Genes and Functional Analysis of AtHB-12, a Putative Hoemobox-leucine Zipper Transcription Factor

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Objectives

Yeast is an useful model for studing cellular salt stress signaling response. In the yeast *Saccharomyces cerevisiae*, two signaling cascades mediate osmotic adjustment and appropriate ion homeostasis under NaCl stress; a mitogen-activated protein (MAP) kinase cascade and a calcineurin, Ca^{2+}/Calmoduline-dependent protein phosphatase, regulated cascade. To identify genes that function in plant salinity stress tolerance, we screened plants genes that cause functional sufficiency for salt tolerance or complement the phenotype of salt sensitive yeast mutants. Because this approach utilizes a screen for genes that can function in stress tolerance rather than a screen for genes whose expression is regulated in response to stress imposition, there is greater likelihood to identify genes that have substantial impact on salt adaptation.

Material and Method

1. Plant cDNA library in yeast expression vector
2. Complementation assay of the yeast mutant
3. β-galactosidase assay using the PMR2A::*LacZ* construct
4. Northern blot analysis

Result and discussion

1. After transforming *Arabidopsis thaliana* cDNA library to yeast cells, we screened plant genes that can rescue the salt-sensitive phenotype of yeast. Through this screening, we got total 34 of salt-tolerant colonies. After sequencing, these cDNAs turned out correspond to 9 kinds of different genes.
2. Among the repertoires of genes we have isolated, we characterized AtHB-12 in detail. Expression of *AtHB*-12 in cnb1 mutant strain conferred tolerance to the NaCl stress but not KCl and sobitol stresses. These results indicate that AtHB-12 is required for Na+ toxicity response.
3. When AtHB-12, a homeobox-leucine zipper transcription factor, was introduced an yeast strain that contain *PMR2A::lacZ* reporter gene, the yeast strain exhibited increased β-galactosidase activity even without NaCl treatment. This result indicates that AtHB-12 functions in the NaCl stress signal transduction pathway of yeast.
4. Transcriptional level of the AtHB-12 gene in plant is enhanced by NaCl or ABA treatment.

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