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Identification of the Small-Heat Shock Protein Gene (*BcHSP17.6-II*) by cDNA Microarray and Its Molecular Characters

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Objectives

To study the signal transduction pathway that is traggered Hydrogen peroxide, we established the optimal experimental conditions for cDNA microarray. We then cloned and sequenced a large number of H_2O_2-responsive genes isolated from Chinese cabbage cells. Among these clones, a small heat-shock protein (sHSP) was identified as being strongly induced by hydrogen peroxide in all time courses. Here, we characterized the cytosolic class II sHSP, *BcHSP17.6-II*, and discuss the functions for acquisition of stress tolerance in plants.

Materials and Methods

1. Plant materials
   : Chinese Cabbage (*Brassica campestris* L. ssp. *pekinensis*)
   : *Arabidopsis thaliana* suspension culture cell
2. Methods : Molecular Characterization of *BcHSP17.6-II*

Results and Discussion

A cDNA encoding sHSP was isolated from H_2O_2-treated Chinese cabbage seedlings by cDNA microarray hybridization. The deduced amino acid sequence of this clone shares 65-87% identity with sHSPs from various species. Comparison of their amino acid sequences with those of other sHSPs indicated high similarity to the cytosolic class II, so this clone was there after designated *BcHSP17.6-II*. Like most sHSPs, *BcHSP17.6-II* contains conserved C-terminal region and HSP20 domain. To investigate the potential responsiveness *BcHSP17.6-II* gene expression to the stresses, *Arabidopsis* suspension cells were treated with 10 mM H_2O_2 and 37°C over a time course. The level of the *BcHSP17.6-II* transcript increased 3 hr after exposure to exogenous H_2O_2 remained constitutively expressed to 12 hr. Following exposure to heat acclimation treatment, the *BcHSP17.6-II* transcript level also increased 2.5 hr after treatment. These results reveal that *BcHSP17.6-II* may be involved in Chinese cabbage defense mechanism.

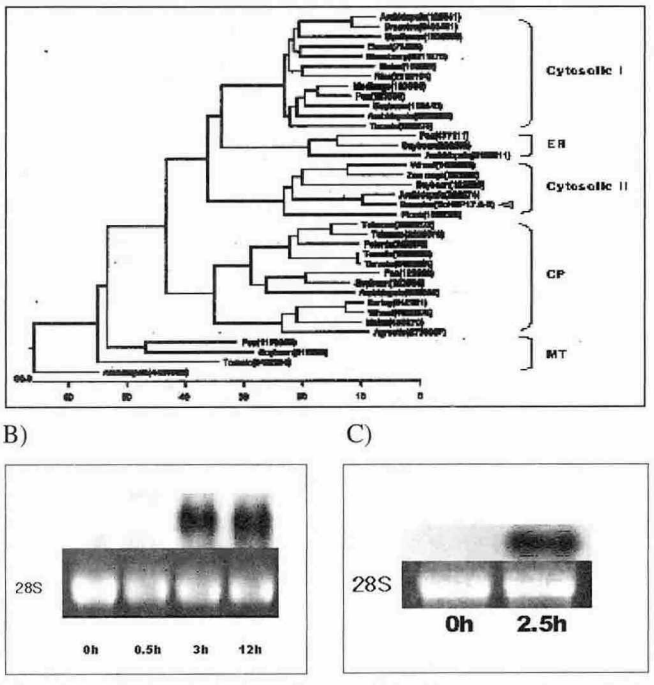

Fig. 1. Analysis of phylogenic tree (A). Northern blot analysis shows that *BcHSP17.6-II* was induced by hydrogen peroxide (B) and heat acclimation (C) in *Arabidopsis thaliana* suspension culture cells.

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