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Sugar Starvation: cue for increased cell wall hydrolases activity

Eun-Jeong Lee and Nozomu Koizumi
Institute of Agricultural Science, Chungnam National University

Objective

We previously identified genes induced by sugar starvation in Arabidopsis, including genes for three genes, putative β-galactosidase (At5g56870), β-xylosidase (At5g49360) and β-glucosidase (At3g60140). They were predicted as secreted proteins that play roles in modification of cell wall polysaccharides form the amino acid similarity. In present study, we attempt to elucidate the molecular mechanisms and functions of these enzymes under sugar starvation.

Materials and Methods

Plant material: Arabidopsis Col-0 cell suspension and whole plants (ecotype Columbia)

Enzyme assay

Protein extraction: by ammonium sulfate precipitation

Enzyme substrates : 4-methylumbelliferyl glycopyranoside

Measurement: spectrofluorometer with the wavelength fixed at 365 and 460 nm

Production of recombinant β-galactosidase and its antibody

Use of recombinant β-galactosidase (At5g56870) protein produced in *E. coli*

***RNA gel-blot analysis and Immunoblot analysis*:** by standard protocols

Determination of cell wall polysaccharides contents

Fraction of cell wall polysaccharides: by Hoson et al. (1995)

Sugar content of each fraction: by gas-liquid chromatography

***Gus staining*:** by standard protocols

Results and Discussion

Three Arabidopsis genes encoding putative β-galactosidase (At5g56870), β-xylosidase (At5g49360) and β-glucosidase (At3g60140) were induced by sugar starvation. Their deduced proteins belong to family 35, 3 and 1 glycosyl hydrolase, respectively. They were predicted as secreted proteins that play roles in modification of cell wall polysaccharides from the amino acid similarity. We hypothesized that those enzymes supply sugar by breakdown of cell wall polysaccharides under sugar starvation. This idea was supported by experiments to measure activities of β-galactosidase, β-xylosidase and β-glucosidase that increased in culture medium of suspension cells by sugar starvation. Secretion of β-galactosidase into the medium was detected by its antibody. Their induction was repressed on suspension cells grown with galactose, xylose and glucose as well as with sucrose. *In planta*, their expression and protein accumulation were detected when photosynthesis was inhibited. Moreover, amount of monosaccharides in pectin and hemicellulose in detached leaves decreased by sugar starvation. These findings suggest that cell wall may function as 'storage of carbon source' in addition to physical support of plant body.

Address : Eun-Jeong Lee(이은정) E-mail : j-le@cnu.ac.kr Tel : 042-821-7822

Figure 1. Induction of gene transcripts under sugar starvation

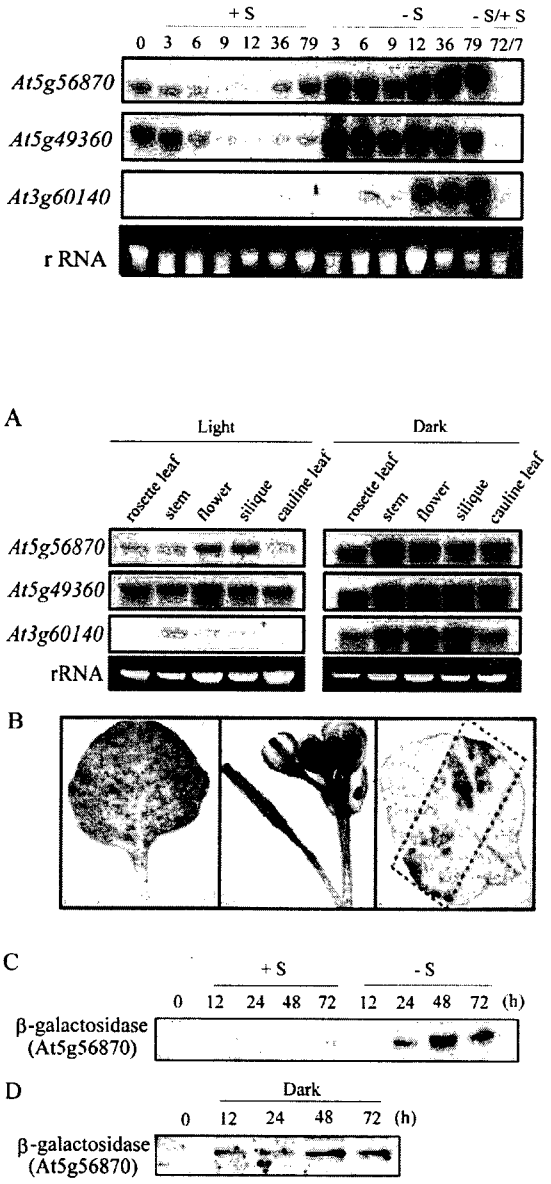

Figure 2. Induction of glycosyl hydrolases in cultured cells by sugar starvation

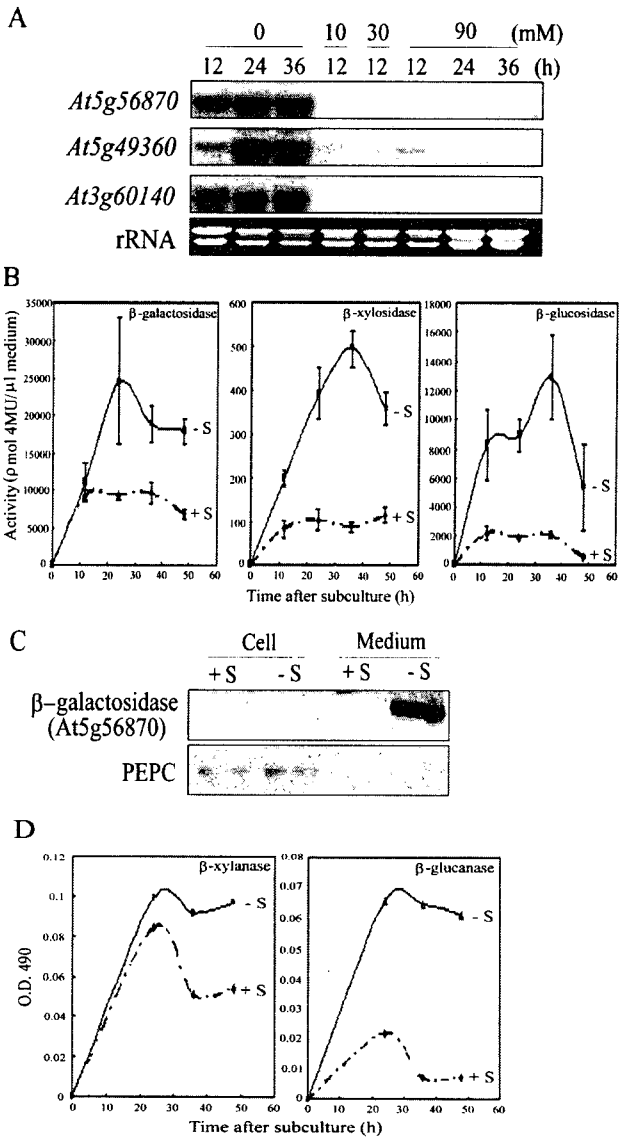

Figure 3. Induction of glycosyl hydrolases in whole plants by dark treatment

Figure 4. Sugar compositions of cell wall polysaccharides in Arabidopsis leaves