SYMP-05

Identification of Sugar-Responsive Genes and Discovery of the New Functions in Plant Cell Wall

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Abstract

The objective of this study is to understand how regulatory mechanisms respond to sugar status for more efficient carbon utilization and source-sink regulation in plants. So, we need to identify and characterize many components of sugar-response pathways for a better understanding of sugar responses. For this end, genes responding change of sugar status were screened using *Arabidpsis* cDNA arrays, and confirmed thirty-six genes to be regulated by sucrose supply in detached leaves by RNA blot analysis. Eleven of them encoding proteins for amino acid metabolism and carbohydrate metabolism were repressed by sugars. The remaining genes induced by sugar supply were for protein synthesis including ribosomal proteins and elongation factors. Among them, I focused on three hydrolase genes encoding putative β-galactosidase, β-xylosidase, and β-glucosidase that were transcriptionally induced in sugar starvation. Homology search indicated that these enzymes were involved in hydrolysis of cell wall polysaccharides. In addition to my results, recent transcriptome analysis suggested multiple genes for cell wall degradation were induced by sugar starvation. Thus, I hypothesized that enzymes for cell wall degradation were synthesized and secreted to hydrolyze cell wall polysaccharides producing carbon source under sugar-starved conditions. In fact, the enzymatic activities of these three enzymes increased in culture medium of *Arabidopsis* suspension cells under sugar starvation. The β-galactosidase encoded by At5g56870 was identified as a secretory protein in culture medium of suspension cells by mass spectrometry analysis. This protein was specifically detected under sugar-starved condition with a specific antibody. Induction of these genes was repressed in suspension cells grown with galactose, xylose and glucose as well as with sucrose.

In planta, expression of the genes and protein accumulation were detected when photosynthesis was inhibited. Glycosyl hydrolase activity against galactan also increased during sugar starvation. Further, contents of cell wall polysaccharides especially pectin and hemicellulose were markedly decreased associating with sugar starvation in detached leaves. The amount of monosaccharide in pectin and hemicellulose in detached leaves decreased in response to sugar starvation. These results supported my idea that cell wall has one of function to supply carbon source in addition to determination of cell shape and physical support of plant bodies.

Introduction

Sugar produced through photosynthesis is used to build up cellular components and for energy source. In addition, sugar is one of the essential signals for plant development, and sugar signaling pathways regulate vital processes accompanied by gene expression. Plants use complex signaling pathways to sense and respond to fluctuations of sugar levels. At same time, sugar signaling pathways are involved in various metabolism and catabolism that produce or utilize carbon. In spite of sugar repression in plants being widely studied using genetic and reverse genetic approaches, knowledge of the regulatory mechanisms of both sugar sensing and responses remains limited compared with that for yeast, presumably due to more complex cellular metabolic pathways.

New insights into regulatory mechanisms of plant sugar sensing have emerged from recent advances in analysis of the Arabidopsis transcriptome (Thimm et al. 2004, Contento et al. 2004, Buchanan-Wollaston et al. 2005). In particular, a number of genes for carbohydrate metabolism, such as genes encoding glycosyl hydrolases, are regulated by sugars. Rice α-amylases, a well-characterized class of glycosyl hydrolases induced by sugar deprivation, catalyze the hydrolysis of starch, which enables remobilization of sugars (Yu et al. 1991, Sheu et al. 1996). Just as α-amylases produce sugars from starch, cell wall glycosyl hydrolases may degrade cell walls to release sugars under sugar-starvation conditions.

However, cell wall polysaccharide-hydrolyzing enzymes have been mainly examined for their roles in fruit softening, seed germination and tissue development. Then, analysis of the global expression profile and characterization of Arabidopsis genes in response to sugars would lead discovery of new cellular processes regulated by sugar.

Results and Discussion

Scatter plot of signal intensity on Arabidopsis macro-arrays

I used cDNA macro-arrays containing approximately 13,000 non-redundant ESTs of *Arabidopsis thaliana* to screen genes whose transcript levels changed in response to sugar status. The signal was normalized to calculate the intensity ratio obtained between 79 h sucrose-starved and 7 h sucrose treatment after 72 h sucrose-starved probes. Analysis of these arrays revealed transcript levels of 184 genes to show 3-fold increase or decrease in response to sugars (Fig. 1a). Subsequently, duplicated sub-arrays were prepared, transcript levels of 73 genes to show 1.5-fold change in comparison with the controls (Fig. 1b)

RNA gel-blot analysis of down-regulated genes to sugar

In order to confirm the result of array analysis, expression profile of the 73 genes detected in the arrays were further examined by RNA blot analysis. Twenty-four genes were repressed by sucrose depletion (data not shown). About half of them were found to encode ribosomal proteins. The remaining included genes for proteins involved in protein synthesis, amino acids

metabolism, secondary metabolism, and energy metabolism. I also identified eleven genes whose transcripts were induced by sucrose depletion. Three genes encoded putative glycosyl hydrolases, which are predicted to function in degradation of cell wall components (Fig. 2).

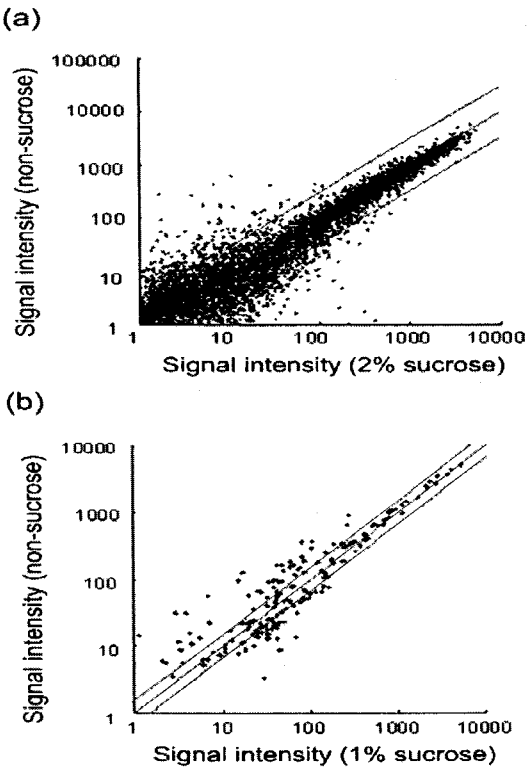

Fig. 1. Identification of sugar-responsive genes

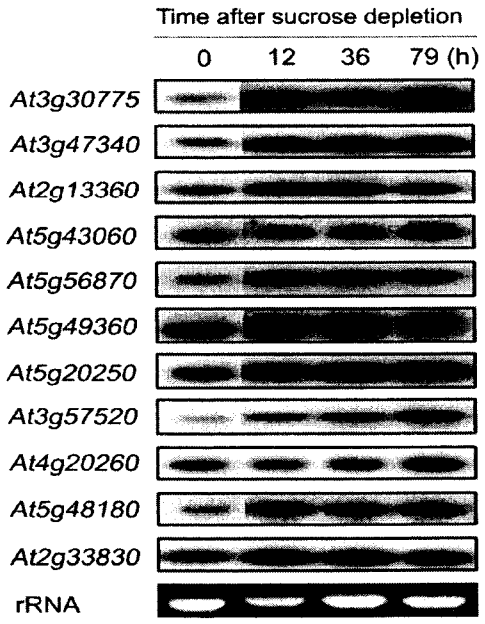

Fig. 2. RNA gel-blot analysis

Three genes encoding possible apoplastic hydrolases

Genes encoding putative β-galactosidase, β-xylosidase, and β-glucosidase were identified to be induced by sugar starvation. The predicted proteins derived from β-galactosidase, β-xylosidase, and β-glucosidase have a potential signal peptide and several potential *N*-glycosylation sites. They also contain the glycosyl hydrolase family domain. Homology search indicated that they are similar to cell wall-degrading enzymes. Taken together, three genes are considered to encode apoplastic hydrolases (Fig. 3).

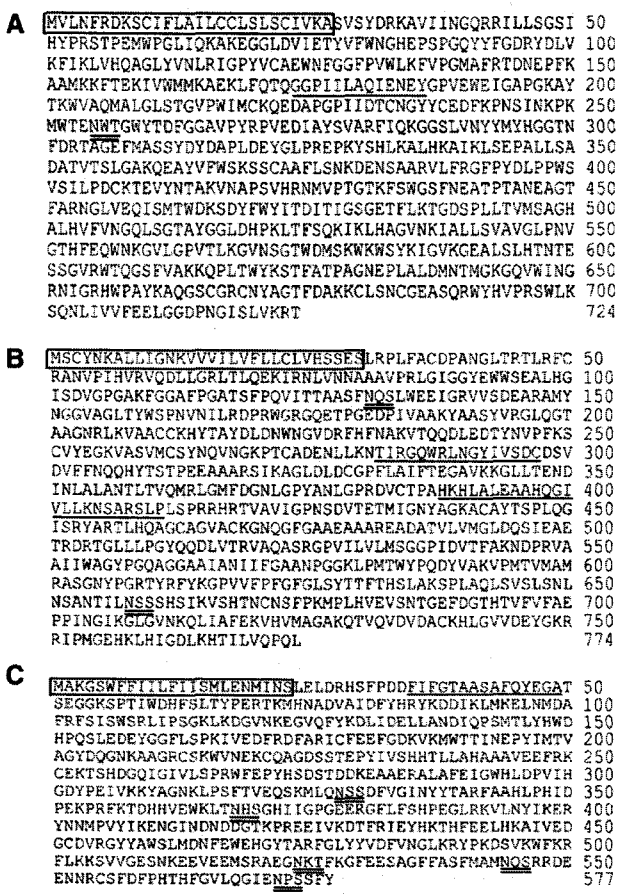

Fig. 3. Deduced amino acid sequences of the three glycosyl hydrolases. (a) ß-galactosidase (At5g56870), (b) ß-xylosidase (At5g49360), and (c) ß-glucosidase (At3g60140).

Characterization of cell wall-degrading enzymes in cultured cells

When sugar was omitted from medium of suspension culture, transcripts of three genes were clearly induced. This induction was completely suppressed by addition of 10 mM sucrose (Fig. 4). Subsequently, to investigate the relation of hexokinase in sugar signaling pathway, the effect of various sugars on expression of these genes were examined. Several hexoses similarly repressed expression of β-galactosidase, β-xylosidase, and β-glucosidase. However, neither 2-deoxy-glucose nor 3-*O*-methyl-glucose affected on expression of these genes, indicating that hexokinase was not involved in their regulation (Fig. 5).

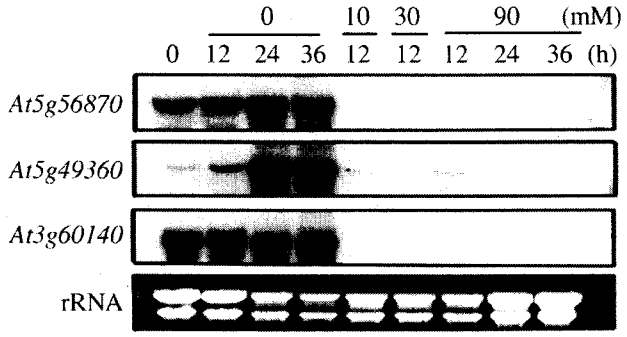

Fig. 4. Expression of three genes encoding glycoside hydrolase

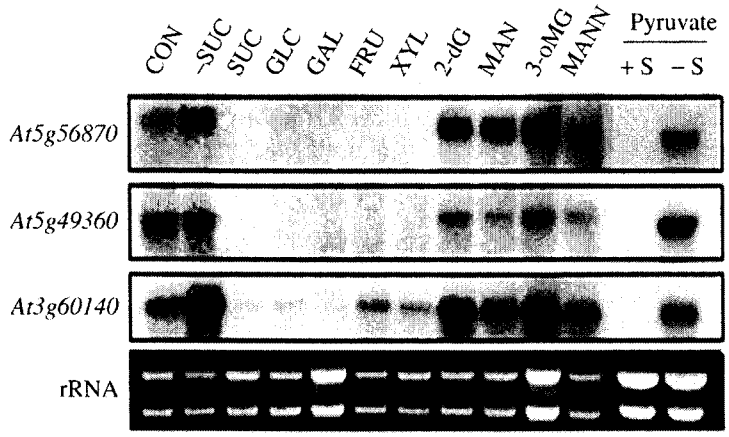

Fig. 5. Effects of carbohydrate metabolites on transcript accumulation.

Hydrolase activities increased by sugar starvation in cultured cells

Activities of β-galactosidase, β-xylosidase, and β-glucosidase in culture medium were measured using synthetic substrates. As shown in Fig. 6, the activities of all three hydrolase in culture medium of suspension cells increased when sucrose was depleted from medium. Also, the presence of β-galactosidase proteins in culture medium depleted with sucrose for 48 h was confirmed with specific antibodies. Protein was not detected when cells were cultured with sucrose (Fig. 7).

Induction of the β-galactosidase in leaves by sugar starvation

Transcripts of the β-galactosidase rapidly decreased when detached leaves were floated on sucrose solution. In contrast, they rapidly increased when leaves were in just water (Fig. 8). Proteins of the β-galactosidase apparently accumulated when sucrose was depleted reflecting accumulation of transcripts (Fig. 8). However, accumulation was much slower than that of transcripts. Proteins were considered to function after 48 h since accumulation was detected at this time point.

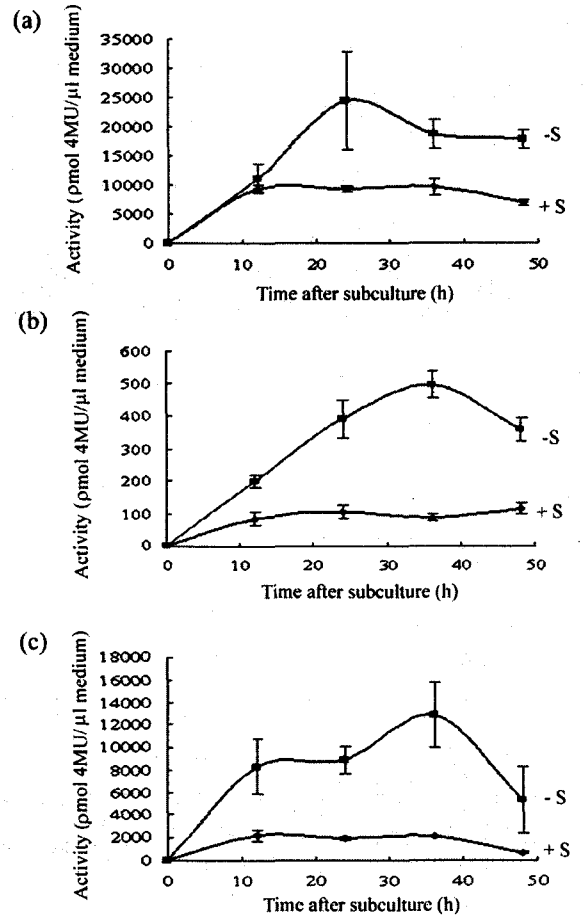

Fig. 6. Enzymatic activities during sugar starvation (a) ß-galactosidase, (b) ß-xylosidase, and (c) ß-glucosidase.

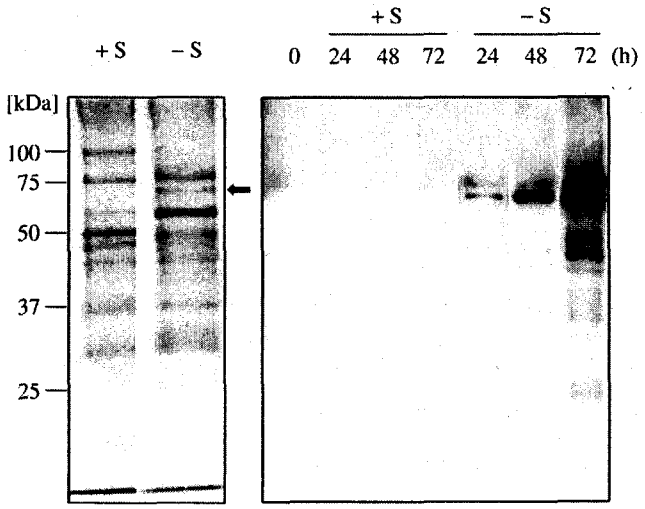

Fig. 7. Identification of secretion of the ß-galactosidase protein.

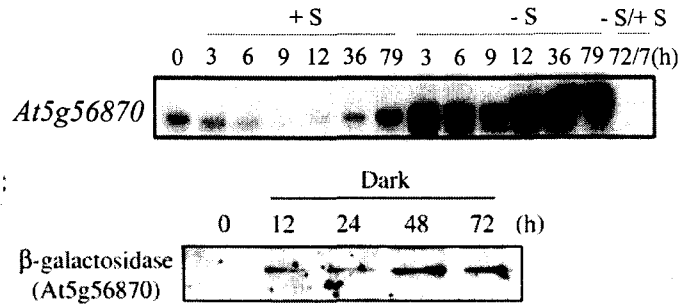

Fig. 8. Induction of ß-galactosidase (RNA-gel blot analysis and immuno-blot analyis)

Effect of various sugars on cell growth

Cultured cells were incubated in media containing indicated carbon compounds at 90 mM in each treatment, or no sugar supplement. After 7 days incubation, wet fresh weight was estimated. Suspension cultures were successfully grown in all of the sugars tested (data not shown).

Induction of β-galactosidase by inhibition of photosynthesis

These results indicated that β-galactosidase is induced in intact plants under natural condition when photosynthesis is inhibited for certain period. Various organs were assayed after plants were kept in darkness for 24 h to induce natural sugar starvation. Transcripts of Gal were increased in all organs when subjected to sugar starvation (Fig. 9a). As known well, starch content decreased by shading. In contrast, GUS activity of transgenic plants harboring a chimeric genes consisting of β-galactosidase and GUS reporter gene clearly increased in leaves where light was shaded (Fig. 9b).

Decrease of cell wall polysaccharides by sugar starvation

The sugar contents of pectin, hemicellulose I and hemicellulose II fractions were significantly lower in leaves incubated in just water than those with sugar. Incubation was conducted under dark condition for 48 h since protein accumulation of β-galactosidase was detected after 48 h of sugar starvation. The sugar content of each fraction was determined with the gas chromatography. The major sugars (Xyl, Gal and Glu) in each fraction decreased suggesting that those were released form cell walls by hydrolase analyzed in the present study (Fig. 10).

Conclusion

According to the result that genes encoding apoplastic hydrolases were induced by sugar starvation, I propose that these enzymes, β-galactosidase, β-xylosidase, and β-glucosidase, are secreted into apoplasts to break down cell wall materials to release a galactose, xylose and glucose as a carbon source that are recycled after incorporation into cells (Fig. 11). In fact,

these enzymes were secreted and cell wall components decreased associating with sugar starvation suggesting our hypothesis is correct. Importance of this metabolic pathway for plant life will be necessary to be clarified.

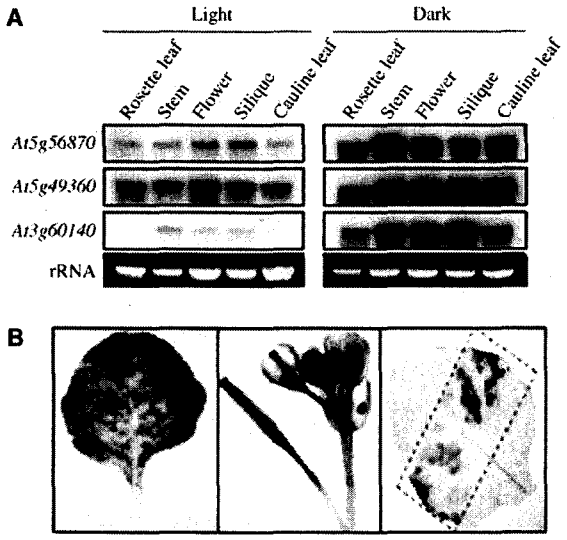

Fig. 9. Induction of glycosyl hydrolases in whole plants by dark treatment. (a) Expression of three genes in various organs. (b) Histochemical localization of GUS activity in transgenic Arabidopsis plants.

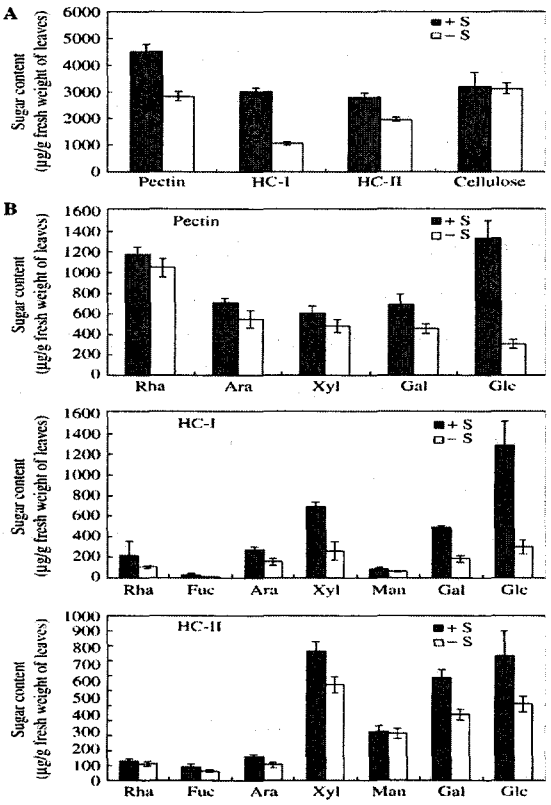

Fig. 10. Sugar composition of cell wall polysaccharides in Arabidopsis leaves.

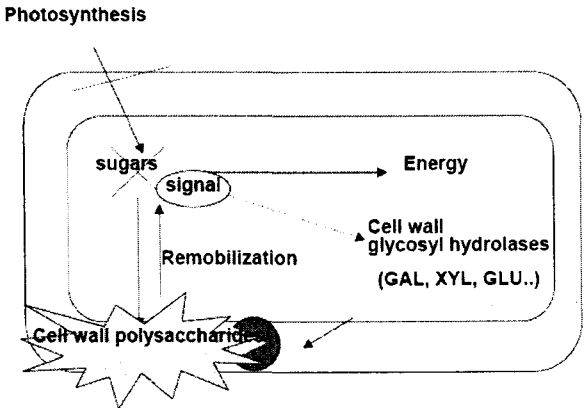

Fig. 11. Model

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