International Journal of Plant Production 2 (4), October 2008
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.

GUASNR
www.ijpp.info

Microarray analysis of gene expression patterns in *Arabidopsis* seedlings under trehalose, sucrose and sorbitol treatment

M. Aghdasi[a], S. Smeekens[b], H. Schluepman[b]

[a]*Departement of Biology, Faculty of Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran.*
[b]*Department of Molecular Plant Physiology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.*
Corresponding author. Email:Aghdasi@gau.ac.ir

Received 25 Feb. 2008; Accepted after revision 8 June 2008; Published online September 2008

Abstract

Trehalose is the non-reducing alpha-alpha-1, 1-linked glucose disaccharide. The biosynthesis precursor of trehalose, trehalose-6-phosphate (T6P), is essential for plant development, growth, carbon utilization and alters photosynthetic capacity but its mode of action is not understood. In the current research, 6 days old seedlings of *Arabidopsis thaliana* (Columbia ecotype) were grown in liquid culture containing 100 mM trehalose, sorbitol or sucrose for 24 hours. Changes in the genes expression patterns were studied by cDNA microarray analysis. In sucrose treatment expression of 1745 genes was significantly changed. But trehalose changed significantly the expression of only 162 genes compared to sorbitol after 24h treatment. Gene expressions profiles revealed that 100 mM trehalose altered 5% of the genes which are changed by 100 mM sucrose. Statistical analysis showed that only 4 genes which are induced by trehalose repressed by sucrose. Exogenous trehalose treatment did not down-regulate the expression of carbon catabolite genes, but up-regulates a specific combination of genes known from biotic stress responses. Trehalose induced gene expression responses related to ROS and secondary metabolism activation. The expression profile shows particularly up-regulation (8-fold) of a glutathione transferase (GST22) under trehalose but not sucrose. Also, trehalose treatment induced expression of the JA and ethylene signaling pathways factors. These findings revealed that trehalose or its precursor, T6P, are important in gene expression regulation of plants.

Keywords: Trehalose; T6P; *Arabidopsis thaliana*; Microarray; Gene expression

Introduction

Trehalose (α-D-glucosyl-[1, 1]-α-D-glucopyranoside) is a non-reducing disaccharide sugar which consists of two glucose units joined by an α-1, 1 linkage. Trehalose is common in nature (Elbein, 1974) and present in a wide variety of organisms (Elbein et al., 2003).

The presence of trehalose had not been reported in plants except in some unusual desiccation-tolerant plants, such as *Selaginella lepidophylla* and *Myrothamnus abellifolia*, where trehalose is accumulated in large quantities apparently as stress protectant (Muller et al., 1995). The inability to detect trehalose in plants led to the suggestion that the majority of higher plants had lost the ability to produce it (Crowe et al., 1992). However, this view changed in 1997. In an attempt to enhance the stress tolerance of crop plants, researchers genetically engineered tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*) to synthesize trehalose (Goddijn et al., 1997). Surprisingly, trace levels of trehalose could be detected in wild type as well as transgenic plants (Goddijn et al., 1997) suggesting that the occurrence of trehalose may be more widespread in plants than previously thought. Trehalose is used as a source of energy and carbon and as protectant in a wide variety of stress. It was shown that trehalose stabilizes proteins and membranes under stress conditions (Crowe et al., 1992).

There are five biosynthetic pathways for trehalose. The most widely reported and the best studied pathway for the biosynthesis of α, α 1, 1-trehalose is that involving the enzyme trehalose-phosphate synthase (TPS). Synthesis of trehalose in plants is typically via its phosphorylated intermediate, trehalose-6-phosphate (T6P). Trehalose-6-phosphate synthase (TPS) converts UDP-Glucose and Glucose-6-phosphate to T6P. Trehalose phosphate phosphatase (TPP) de-phosphorylates T6P to trehalose. Trehalase cleaves trehalose to two glucose molecules (Elbein, 1974).

Externally applied trehalose causes dramatic effects on plant growth and carbon allocation. Trehalose feeding to Arabidopsis seedlings leads to accumulation of starch and trehalose -6-phosphate (T6P), enhance AGPase activity and inhibition of shoot and root growth (Wingler et al., 2000; Fritzius et al., 2001; Kolbe et al., 2005). Recently, it was proposed that not trehalose but rather than the intermediate (T6P) causes the observed effects on growth and carbon metabolism (Schluepmann et al., 2004). Although it is clear that T6P has dramatic effects on plant metabolism, growth and development, little is known about the exact mechanism of its action (Schluepmann et al., 2004; Van Dijken et al., 2004; Pellny et al., 2004).

T6P is an important key regulatory molecule that might control growth and development in specific pathway. Current documents provide a specific role for T6P to control sugar metabolism. It was reported that over-expressing of *E.coli TPS* in tobacco plants increase T6P level and photosynthesis per unit leaf area under saturating light, whereas expressing *E.coli TPP* have reduced photosynthetic rates (Pellny et al., 2004).

Exogenous trehalose induced the expression of sucrose: fructose-6-fructosyl-transferase in barley leaves (Muller et al., 2000), the large subunit of adenosine diphosphate (ADP)-glucose pyrophosphorylase in Arabidopsis (Wingler et al., 2000), and increased sucrose synthetase and alkaline invertase activities but concomitantly reduced acid invertase activity in soybean roots (Muller et al., 1998). Also adding 30 mM trehalose to Arabidopsis seedlings altered transcript level of transcription factors, cell wall modification, nitrogen metabolism and fatty acid biosynthesis genes (Bae et al., 2005).

The general goal of this research was to uncover the targets of T6P. In this study, comprehensive analyses of gene expression in response to 100 mM sucrose and trehalose treatments were conducted using the 8K gene chip from Affymetrix. With this method, we identified several stress-related genes in response to 100 mM trehalose treatment

Methods and materials

Plant materials and growth conditions

Seeds of *Arabidopsis thaliana* accession Columbia were sterilized 5 minutes with 70% Ethanol followed by 10 minutes in 20% commercial bleach (4% w/v chlorine) and washed 5 times in sterile milli-Q water. Seeds were stratified in darkness at 4°C for 2 days.

Seedlings were grown for 6d in shaking liquid half strength MS medium, (140 rpm). Sugars were added for 24h before harvest to a final concentration of 100 mM. Frozen tissue was ground using two 3-mm diameter glass beads in Eppendorf tubes with a Dismembranator (Braun, Melsungen, Germany) and RNA was extracted from seedlings with Plant Mini kit (Qiagen, Hilden, Germany). To anneal probes to Affymetrix microarrays, RNA concentrations were adjusted to 25 μg RNA per labeling reactions using both the photo spectrometric and capillary electrophoresis methods (RNA Lab-on-a-chip from Caliper Technologies, Mountain View, CA). All data was analyzed statistically using the R language environment for statistical computing (http://www.r-project.org) version 2.2 and Bioconductor release 1.7 (http://www.bioconductor.org). Differential expressed genes were identified using the LIMMA package (Smyth, 2004). Data was normalized using the VSN package (Huber et al., 2002) and linearly fitted using the recommendations of the LIMMA vignette. Normalization of data using the Loess method showed similar results (data not shown), because of variances were not equal across hybridizations (sucrose had a major effect compared to trehalose that had a more limited effect when compared to sorbitol in the experiment), VSN normalization will reflect the better situation *in planta* (Freudenberg et al., 2004). Visual examination of raw and normalized distributions by diagnostic plots confirmed the conclusion. A posterior residual standard deviation was employed (Smyth, 2004) independently for each treatment and the interesting contrasts (change in the trehalose treatment compared to sucrose). The obtained P-values were corrected for multiple testing errors using the BH procedure (Benjamini and Hochberg 1995), yielding q values. Lists of q-values were transferred to Microsoft Excel™ and sorted for maximal trehalose effect. The GST sequences were aligned to the Tair6 gene model database of transcripts. Genes were classified as differentially expressed if they showed significantly changes (q V 0.05) in trehalose or sucrose compared to sorbitol. To compare the differentially expressed genes with other Microsoft Excel™ was used.

Results

Specific changes in seedling gene-expression after trehalose treatment

Current data is belong to the results of the LIMMA and VSN packages as described in Materials and Methods showed in Figure 1. Whilst sucrose treatment leads to 1745 genes with significantly changed expression, trehalose leads to only 162 genes with significantly changed gene expression compared to sorbitol after 24h. The changes under trehalose treatment range from the 4 fold down-regulation of At3G60140, a glycosyl hydrolase family protein, up to 38 fold up-regulation of *WAK1*. 60% of the genes affected by trehalose are also affected by sucrose: of the 113 trehalose up-regulated genes, 57 are also

up-regulated by sucrose, and of the 49 genes down-regulated by trehalose 40 are also down-regulated by sucrose treatment. Seedlings grow on sucrose but fail to develop on trehalose medium. This could be due to trehalose induction of sucrose-induced genes during a lack of metabolisable carbon.

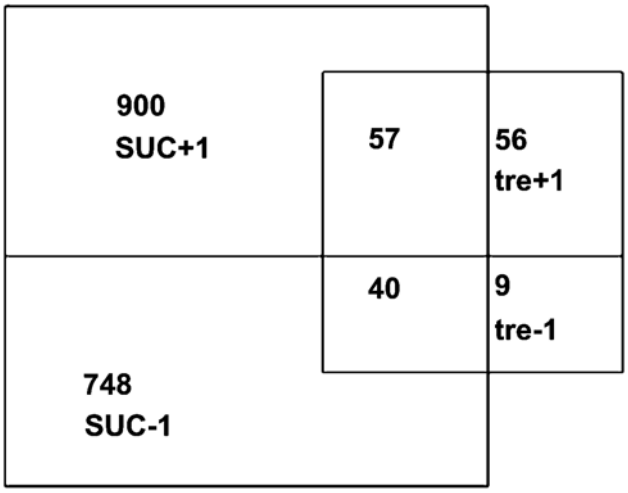

Figure 1. Genes expressed specifically by trehaloseDiagram of sugar induced gene-expression compared to sorbitol treatment; numbers refer to the number of genes with significant change; +suc, up-regulated by sucrose; -suc, down-regulated by sucrose; +tre, up-regulated by trehalose; -tre, down-regulated by trehalose.

A list of genes that respond to trehalose and sucrose in a similar way is provided in Table 1. Alternatively, up or down-regulation of genes which are important for growth in sucrose no longer occurs on trehalose and thus development is inhibited. A list of genes differentially regulated by sucrose and trehalose is provided in Table 2.

Statistical analysis failed to uncover genes that are down-regulated by trehalose but up-regulated by sucrose, but it identified 4 genes that are up-regulated by trehalose but down-regulated by sucrose. These genes include *TPPF* (At4g12430), a homologues to *AtTPPB*, *PRO1*; the mitochondrial proline oxidase stress responsive gene, a protein related to the vacuolar calcium binding protein encoded by At1g62480 and a protein related to the bacterial TOLB encoded by At4g01870. *TPPF* up-regulation suggests that T6P accumulation under trehalose treatment is sensed. Genes that respond to trehalose and remain unchanged by sucrose confirms this (Table 3): *TPPG* encoded by At4g22590, *TPPH* encoded by At4g39770 and *AtTPPB* are up-regulated 5.8, 2.7 and 2.3 fold, respectively after trehalose treatment. In addition, the up-regulated trehalose specific gene reflects a specific stress response. In Table 2, *PRO1* is up-regulated by trehalose while it is down-regulated by sucrose; in Table 3, up-regulation of glutathione S-transferase, *TSA1*, *HSP17.7-CII* and *HSP17.4-CI*, *SAG102*, *PAD4*, anthranilate phophoribosyltransferase, sulfate adenylyltransferase3, *PDF2.3* and several peroxidases is further indicative of oxidative stress and induction of secondary metabolite pathways. Five fold up-regulation of the ethylene response factor *AtERF-2* and 4-fold up-regulation of the coronatine induced

protein1 further reveal likely involvement of JA and ethylene signal transduction pathways. Surprisingly, some genes that respond specifically to trehalose but not to sucrose are known in biotic interactions: AvrRpt2-induced *AIG2* (4 fold up-regulated), *EDS5* (4 fold up-regulated), *PAD4* (4 fold up-regulated) and *WAK2* (2.5 fold up-regulated). In addition genes induced by both sucrose and trehalose, such as, *WAK1* (38 fold up-regulated), *EDS1* (3 fold), suggestive of trehalose mediated priming as a specific disease response.

Table 3B also showed down-regulation of *GASA1, MERI5B* and *EXGT-A3*, and *SEX1* under trehalose but not under sucrose. Down-regulation of *SEX1* was also found by Ramon *et al*., 2007 in seedlings growing on the combination of 30 mM trehalose and 10 µM Validamycin. Down- regulation of *GASA1, MERI5B* and *EXGT-A-3* may affect cell-elongation processes in roots and hypocotyls of seedlings grown on 100 mM trehalose.

ICL is present on this 8K chip and the gene is seemingly catabolite repressed on sucrose but repression is not significant on trehalose (not shown). Table 4 shows expression of genes typically down-regulated by carbon catabolite repression on sucrose; Table 4 reveals that trehalose is unable to raise catabolite repression as sucrose does. For example, from the 41 genes listed in Table 4 that show more than 5-fold down-regulation by sucrose, only 4 genes are also repressed by trehalose: carbonic anhydrase 1 and 2 (2.5-fold each), *STP1* glucose transporter1 (3-fold) and asparagine synthetase2 (3.5-fold). *STP1* was previously found to be down-regulated by the combination of 30 mM trehalose and 10 µM validamycine (Ramon et al., 2007). In addition *MYB75* is 4-fold up-regulated by sucrose but not by trehalose, again suggesting that trehalose is not just acting as a sucrose analogue on sucrose sensing pathways.

Table 1A. Genes up-regulated in response to trehalose and sucrose. Seedlings were grown in shaking liquid medium in continuous light then supplied for 24h with 100 mM of sorbitol, sucrose or trehalose before harvest and RNA extractions. The Affymetrix 8K gene chip was used for transcriptional profiling, and normalization and data analysis were as described in Materials and Methods. Probe set, probe set identity; A, average expression over the experiment (log2); suc, change after sucrose treatment compared to sorbitol treatment (log2); tre, change after trehalose treatment compared to sorbitol treatment (log2). P value suc, P value for the change after Sucrose; P value tre, P value for the change after trehalose; Locus, AGI number of the gene detected with the probe set; description, abbreviated TAIR annotation dating from February 2007.

Probe set NA	A	suc	tre	p.value suc	p.value tre	Locus	description
15616_s_at	9,5	5,0	5,2	9,1E-09	6,2E-09	AT1G21250	wall-associated kinase 1 (WAK1)
12879_at	8,0	2,5	4,8	4,9E-07	6,4E-09	AT1G33960	avirulence-responsive protein / avirulence induced gene (AIG1)
13217_s_at	7,4	6,8	5,1	4,9E-09	3,2E-08	AT3G50770	calmodulin-related protein, putative, similar to regulator of gene silencing calmodulin-related protein
17917_s_at	9,3	5,3	3,7	4,8E-09	5,7E-08	AT2G41090	calmodulin-like calcium-binding protein, 22 kDa (CaBP-22),
15431_at	9,0	2,2	2,0	1,6E-06	3,1E-06	AT4G27280	calcium-binding EF hand family protein
12521_at	9,2	4,0	2,4	1,0E-07	3,3E-06	AT3G51860	cation exchanger, putative (CAX3)
20323_at	6,5	2,7	2,4	1,6E-06	3,3E-06	AT2G29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI)
16638_at	5,7	2,1	2,1	3,8E-06	4,5E-06	AT3G28210	zinc finger (AN1-like) family protein
18003_at	9,8	2,5	1,5	1,5E-07	4,6E-06	AT1G72930	Toll-Interleukin-Resistance (TIR) domain-containing protein
20653_s_at	6,7	1,2	1,6	5,4E-05	8,3E-06	AT3G48090	disease resistance protein (EDS1)
13177_at	8,4	1,2	2,0	2,4E-04	8,9E-06	AT4G12720	MutT/nudix family protein
16465_at	9,2	2,2	1,8	3,7E-06	1,1E-05	AT5G02490	heat shock cognate 70 kDa protein 2 (HSC70-2) (HSP70-2)
13279_at	7,1	1,4	2,2	2,6E-04	1,1E-05	AT5G12020	17.6 kDa class II heat shock protein (HSP17.6-CII)
18672_s_at	9,8	1,6	1,5	5,7E-06	1,3E-05	AT1G27770	calcium-transporting ATPase 1
20018_at	7,1	1,8	1,7	8,3E-06	1,5E-05	AT2G44300	lipid transfer protein-related
15483_s_at	5,8	2,2	2,9	1,0E-04	1,6E-05	AT2G46650	cytochrome b5
17104_s_at	10,2	2,2	1,7	2,6E-06	1,7E-05	AT4G35630	phosphoserine aminotransferase, chloroplast (PSAT)
14984_s_at	5,4	1,5	1,1	4,8E-06	3,1E-05	AT4G27560; AT4G27570	glycosyltransferase family protein

Table 1 A.

15629_s_at	8,9	4,4	2,4	5,2E-07	3,3E-05	AT1G17745	D-3-phosphoglycerate dehydrogenase / 3-PGDH,
15193_s_at	12,0	1,8	1,6	1,8E-05	4,0E-05	AT2G30870	glutathione S-transferase
13588_at	10,3	2,3	1,5	2,4E-06	4,6E-05	AT4G34200	D-3-phosphoglycerate dehydrogenase, putative / 3-PGDH
12881_s_at	11,4	1,2	1,5	1,9E-04	5,2E-05	AT5G42650	allene oxide synthase (AOS) / hydroperoxide dehydrase / cytochrome P450 74A (CYP74A),
12515_at	7,9	1,6	1,1	5,6E-06	6,2E-05	AT2G39700	expansin, putative (EXP4), similar to alpha-expansin 6 precursor
18591_at	8,3	1,5	1,6	7,7E-05	6,2E-05	AT5G08790	no apical meristem (NAM) family protein, contains Pfam
12500_s_at	8,2	2,9	1,3	3,5E-07	6,8E-05	AT1G51760	IAA-amino acid hydrolase 3 / IAA-Ala hydrolase 3 (IAR3), identical to IAA-Ala hydrolase (IAR3)
20017_at	10,8	2,6	1,6	3,2E-06	7,3E-05	AT2G44290	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein (YLS3)
17092_at	9,1	1,5	1,2	1,5E-05	7,7E-05	AT5G63980	3'(2'),5'-bisphosphate nucleotidase / inositol polyphosphate 1-phosphatase / FIERY1 protein (FRY1) (SAL1),
15211_s_at	8,3	0,8	1,0	3,3E-04	7,8E-05	AT5G27380	glutathione synthetase (GSH2)
13641_at	8,9	1,3	1,6	3,5E-04	8,6E-05	AT4G33300	disease resistance protein (CC-NBS-LRR class)
17600_s_at	6,4	1,6	1,5	6,1E-05	9,0E-05	AT5G42650	allene oxide synthase (AOS) / hydroperoxide dehydrase / cytochrome P450
18585_at	7,4	0,8	1,0	3,8E-04	9,7E-05	AT5G53130	cyclic nucleotide-regulated ion channel / cyclic nucleotide-gated channel (CNGC1),
16545_s_at	5,3	1,0	1,1	2,1E-04	1,0E-04	AT1G19850	transcription factor MONOPTEROS (MP) / auxin-responsive protein (IAA24) / auxin response factor 5 (ARF5
14686_at	8,2	1,1	1,3	2,7E-04	1,1E-04	AT1G09970	leucine-rich repeat transmembrane protein kinase, putative, Similar to A. thaliana receptor-like protein kinase
19833_s_at	9,9	0,9	1,0	3,1E-04	1,2E-04	AT5G04590	sulfite reductase / ferredoxin (SIR)
16916_s_at	7,1	2,9	2,0	1,2E-05	1,2E-04	AT5G02490	heat shock cognate 70 kDa protein 2 (HSC70-2) (HSP70-2)
14032_at	7,9	2,7	1,7	5,4E-06	1,4E-04	AT4G37370	cytochrome P450
12002_at	8,2	2,4	1,0	5,4E-07	1,5E-04	AT4G02940	oxidoreductase, 2OG-Fe(II) oxygenase family protein
14704_s_at	6,3	1,6	1,4	5,4E-05	1,5E-04	AT2G14560	expressed protein
18561_at	8,9	1,7	0,9	1,8E-06	1,7E-04	AT5G12140	cysteine protease inhibitor, putative / cystatin, putative
15196_s_at	5,6	1,5	1,2	5,2E-05	1,8E-04	AT4G04610	5'-adenylylsulfate reductase (APR1) / PAPS reductase homolog (PRH19),
18010_s_at	8,5	1,6	1,1	1,7E-05	1,9E-04	AT3G54110	plant uncoupling mitochondrial protein (PUMP)
18735_s_at	9,7	0,9	0,9	1,5E-04	2,0E-04	AT4G23100	glutamate-cysteine ligase / gamma-glutamylcysteine synthetase (GSH1)
14882_at	4,8	1,3	1,2	1,1E-04	2,1E-04	AT4G39670	expressed protein
19664_at	5,9	1,2	1,1	1,5E-04	2,5E-04	AT4G39230	isoflavone reductase
12883_at	8,2	1,8	0,8	1,3E-06	2,5E-04	AT4G08390	L-ascorbate peroxidase, stromal (sAPX)
17781_at	5,1	2,6	1,0	6,4E-07	2,6E-04	AT3G47780	ABC transporter family protein
15846_at	6,4	1,1	1,0	1,3E-04	2,6E-04	AT2G14560	expressed protein
17109_s_at	9,4	1,4	1,0	2,9E-05	3,0E-04	AT3G03640	glycosyl hydrolase family 1 protein
13834_at	6,1	1,2	1,2	2,9E-04	3,4E-04	AT4G01010	cyclic nucleotide-regulated ion channel
18594_at	9,2	1,6	0,8	4,1E-06	3,6E-04	AT1G01470	late embryogenesis abundant protein
15668_s_at	11,1	2,4	0,8	2,7E-07	3,6E-04	AT4G11010	nucleoside diphosphate kinase 3, mitochondrial (NDK3
14573_at	6,0	1,9	0,9	3,5E-06	3,9E-04	AT4G02360	expressed protein
18596_at	6,2	3,7	1,0	8,5E-08	4,0E-04	AT1G62570	flavin-containing monooxygenase family protein / FMO family protein
13282_s_at	6,7	5,8	1,2	1,5E-08	4,4E-04	AT4G25200	23.6 kDa mitochondrial small heat shock protein (HSP23.6-M)
14720_s_at	8,4	1,0	0,9	3,3E-04	5,0E-04	AT1G35720	annexin 1 (ANN1), identical to annexin (AnnAt1)
16462_s_at	8,6	1,6	0,7	2,9E-06	5,1E-04	AT2G38380	Peroxidase 22 (PER22)(P22)(PRXEA)/basic peroxidase E

Table 1 B. Genes down-regulated by trehalose and sucrose.

Probe set NA	A	suc	tre	p.value suc	p.value tre	Locus	description
13645_at	8,0	-1,7	-1,8	1,3E-05	1,1E-05	AT1G05340	expressed protein
12574_at	5,6	-3,5	-2,1	4,2E-07	1,4E-05	AT3G60140	glycosyl hydrolase family 1 protein
16422_at	9,2	-5,0	-1,7	1,0E-08	1,6E-05	AT2G33830	dormancy/auxin associated family protein
16488_at	8,9	-4,3	-1,6	2,9E-08	2,2E-05	AT1G11260	glucose transporter (STP1)
15122_at	11,9	-2,1	-1,2	9,7E-07	4,2E-05	AT3G16240	delta tonoplast integral protein (delta-TIP)
15422_at	7,1	-3,6	-1,4	1,2E-07	5,0E-05	AT4G04330	expressed protein
20290_s_at	6,6	-1,4	-1,2	2,6E-05	9,3E-05	AT5G25120 AT5G25130	cytochrome P450 family protein
18755_at	7,2	-2,1	-1,3	3,7E-06	1,0E-04	AT4G25780	pathogenesis-related protein, putative, similar to gene PR-1 protein
19734_at	6,8	-1,9	-1,2	6,7E-06	1,0E-04	AT3G26630	pentatricopeptide (PPR) repeat-containing protein
20362_at	7,5	-3,8	-1,2	6,4E-08	1,2E-04	AT1G71030	myb family transcription factor
19453_at	5,1	-4,1	-1,9	9,3E-07	1,4E-04	AT2G22980	similar to serine carboxypeptidase S10 family protein

Table 1 B.

13972_at	5,8	-2,0	-1,1	2,9E-06	1,4E-04	AT4G17810	similar to zinc finger (C2H2 type) family protein
15901_at	7,4	-2,6	-0,9	2,1E-07	1,7E-04	AT1G54740	expressed protein
14737_s_at	10,1	-3,9	-1,2	6,3E-08	1,9E-04	AT2G13360	serine-glyoxylate aminotransferase-related
14386_at	7,9	-2,8	-1,2	6,5E-07	2,1E-04	AT2G47910	expressed protein
12389_at	5,9	-1,2	-1,0	4,5E-05	2,1E-04	AT1G78720	protein transport protein sec61
19759_at	8,3	-1,5	-0,8	5,4E-06	2,4E-04	AT1G23020	ferric-chelate reductase
16648_at	8,4	-2,5	-0,9	1,9E-07	2,6E-04	AT5G20240	floral homeotic protein PISTILLATA (PI)
16141_s_at	8,6	-1,0	-0,8	5,3E-05	3,0E-04	AT1G58360	amino acid permease I (AAP1)
18484_at	8,4	-1,4	-0,9	2,5E-05	3,2E-04	AT4G37760	squalene monooxygenase
14930_at	7,7	-2,3	-0,8	2,2E-07	3,3E-04	AT2G21530	forkhead-associated domain-containing protein
16490_at	8,3	-2,7	-0,9	2,0E-07	3,4E-04	AT2G18280	tubby-like protein 2 (TULP2)
20344_at	6,5	-2,7	-1,3	3,7E-06	3,7E-04	AT2G15090	fatty acid elongase
13706_at	6,1	-4,2	-1,2	1,1E-07	3,7E-04	AT2G18700	glycosyl transferase family 20 protein / trehalose-phosphatase family protein, similar to trehalose-6-phosphate synthase SL-TPS/P
12768_at	9,2	-2,9	-1,0	3,9E-07	3,8E-04	AT2G15890	expressed protein
16428_at	9,3	-5,4	-1,4	4,5E-08	3,9E-04	AT3G01500	carbonic anhydrase 1
15144_s_at	8,6	-4,3	-1,3	1,8E-07	4,0E-04	AT5G14740	carbonic anhydrase 2
13803_at	7,0	-3,0	-1,0	3,4E-07	4,1E-04	AT4G16690	esterase/lipase/thioesterase family protein
13382_at	6,6	-1,8	-0,9	3,9E-06	4,1E-04	AT2G42750	DNAJ heat shock N-terminal domain
14387_g_at	6,6	-2,8	-1,2	1,5E-06	4,2E-04	AT2G47910	expressed protein
19545_at	10,0	-3,2	-0,9	9,7E-08	4,4E-04	AT1G54500	rubredoxin family protein
17361_s_at	3,4	-1,3	-1,2	2,9E-04	4,7E-04	AT4G10120	sucrose-phosphate synthase
19451_at	4,6	-2,0	-0,9	2,7E-06	4,7E-04	AT1G61820	glycosyl hydrolase family 1 protein
18976_at	7,9	-2,2	-0,9	1,2E-06	4,7E-04	AT1G08980	amidase family protein
15154_at	8,3	-3,3	-1,8	1,1E-05	4,8E-04	AT3G47340	asparagine synthetase 1
18009_s_at	8,8	-3,2	-1,5	3,1E-06	4,8E-04	AT2G25080	phospholipid hydroperoxide glutathione peroxidase
16210_at	5,4	-2,1	-0,7	4,8E-07	4,8E-04	AT4G04850	K+ efflux antiporter
12785_at	10,5	-2,5	-1,1	3,6E-06	4,9E-04	AT4G33010	glycine dehydrogenase
15576_s_at	8,0	-4,5	-1,8	1,6E-06	5,1E-04	AT2G25900	zinc finger (CCCH-type) family protein
15519_s_at	6,4	-3,3	-1,3	1,6E-06	5,4E-04	AT1G03090	methylcrotonyl-CoA carboxylase alpha chain

Table 2. Genes up-regulated in response to trehalose and down-regulated by sucrose.

Probe set NA	A	suc	tre	p.value suc	p.value tre	locus	description
20570_at	5,62	-1,14	2,26	6,5E-05	7,2E-07	AT4G12430	trehalose-6-phosphate phosphatase, putative, similar to trehalose-6-phosphate phosphatase (AtTPPB) (Arabidopsis thaliana)
15124_s_at	10,29	-1,62	0,85	3,0E-06	1,9E-04	AT3G30775	proline oxidase, mitochondrial / osmotic stress-responsive proline dehydrogenase (POX) (PRO1) (ERD5)
17909_at	9,36	-1,99	1,49	5,5E-05	3,4E-04	AT1G62480	vacuolar calcium-binding protein-related, contains weak similarity to vacuolar calcium binding protein (Raphanus sativus)
13656_at	8,44	-1,49	1,25	1,5E-04	4,3E-04	AT4G01870	tolB protein-related, contains weak similarity to TolB protein precursor (Swiss-Prot:P44677) (Haemophilus influenzae)

Discussion

Genes with an expression specifically controlled by T6P

Microarray expression profiles revealed that carbon catabolite repression is not very detectible after 24h of trehalose compared with sucrose treatment. However, long-term growth on trehalose induced catabolite repression and chlorophyll levels and LHCB1 were considerably lower on trehalose (data not shown). This repression is consistent with a senescence response due to starvation rather than hexose accumulation and catabolite respression (Moore et al., 2003; Lim et al., 2006). Starvation may result from reactions triggered by trehalose or T6P. Alternatively, T6P activates carbon utilization processes in the absence of metabolisable carbon. Microarray expression profiles showed that fed trehalose is not just signaling like a sucrose analogue, far less genes respond to trehalose, compared to sucrose. Possibly, metabolisable sugars like sucrose are also sensed in metabolic steps downstream such as in glycolysis. This was previously found for the

response to carbon and nitrogen that may depend on a metabolite product of carbon and nitrogen assimilation (Gutirrez et al., 2007). Alternatively, trehalose is recognized specifically and induces gene expression responses related with ROS and secondary metabolism activation. The expression profile shows particularly high up-regulation (8-fold) of a glutathione transferase (GST22) on trehalose but not on sucrose (Table 3A). The enzyme is also induced by phytoprostanes that are made upon ROS oxidation of lipids (Leoffler et al., 2005); this suggests presence of oxidative stress upon trehalose treatment and is also consistent with results from Bae et al., 2005. Induction of proline dehydrogenase hypoosmotic shock may be due to the swelling of root cells in the extension zone, and may occur in response to the osmotic stress sustained by these cells, but it does not explain why these cells swell. The explanation for swollen cells of the root extension zone may be due to the starvation of these sink cells that may lead to reduced cell wall stability, in other hand two xyloglucan endotransglycosydases (MERI5B, EXGT-A3), enzymes known to be correlated with elongation processes are down-regulated by trehalose treatment (Table 3B). Interestingly, trehalose specifically induces expression of *EDS1*, *EDS5* and *PAD4,* genes which are salicylic acid defense response while sucrose only up-regulate *EDS1* expression. Trehalose treatment does not induce *PR1* expression, possibly due to missing expression of *NDR1* (Nawrath et al., 2002). Moreover trehalose treatment up-regulates both *WAK1* and *WAK2* while showed sucrose treatment only up-regulates *WAK1*. Results suggest biotic defense activation by trehalose or T6P.

Table 3 A. Genes up-regulated in response to rehalose and unchanged by sucrose.

Probe set NA	A	suc	tre	p.value suc	p.value tre	Locus	description
19640_at	7,3	1,3	3,0	6,9E-04	3,3E-06	AT2G29460	glutathione S-transferase, putative
19883_at	7,8	0,7	2,5	6,7E-04	1,1E-07	AT4G22590	trehalose-6-phosphate phosphatase, putative, similar to trehalose-6-phosphate phosphatase (AtTPPA)
16439_at	2,7	0,8	2,4	1,8E-02	3,8E-05	AT1G31580	expressed protein, identical to ORF1 (Arabidopsis thaliana)
16609_at	5,5	1,0	2,4	7,7E-04	3,8E-06	AT5G47220	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ATERF-2)
14672_at	6,8	1,3	2,3	9,3E-03	4,7E-04	AT3G54640	tryptophan synthase, alpha subunit (TSA1)
18881_at	8,9	0,7	2,2	4,0E-03	2,3E-06	AT1G12080	expressed protein
12880_at	5,9	0,6	2,2	9,6E-02	1,6E-04	AT3G28930	avrRpt2-induced AIG2 protein (AIG2)
13277_i_at	7,4	1,5	2,1	5,7E-04	5,7E-05	AT5G12030	17.7 kDa class II heat shock protein 17.6A (HSP17.7-CII)
15778_at	5,3	0,0	2,0	9,1E-01	5,0E-04	AT3G46090	zinc finger (C2H2 type) family protein (ZAT7)
12916_at	4,5	-0,7	2,0	5,4E-02	2,2E-04	AT1G19670	coronatine-responsive protein / coronatine-induced protein 1 (CORI1)
18228_at	5,6	-0,6	1,8	9,9E-02	5,0E-04	AT3G15356	legume lectin family protein, contains Pfam domain, PF00139: Legume lectins beta domain
17653_at	7,0	0,5	1,8	5,3E-02	1,2E-04	AT4G39030	enhanced disease susceptibility 5 (EDS5) / salicylic acid induction deficient 1 (SID1),
17901_at	7,4	0,9	1,8	6,1E-04	9,1E-06	AT2G44670	senescence-associated protein-related
14249_i_at	7,6	0,6	1,7	8,0E-02	5,3E-04	AT3G52430	phytoalexin-deficient 4 protein (PAD4)
13275_f_at	7,3	1,2	1,7	1,1E-03	1,1E-04	AT3G46230	17.4 kDa class I heat shock protein (HSP17.4-CI)
20547_at	5,7	-0,4	1,7	4,5E-02	1,6E-05	AT5G04950	nicotianamine synthase, putative, similar to nicotianamine synthase (Lycopersicon esculentum)
14620_at	6,4	0,7	1,7	2,4E-02	3,1E-04	AT5G17990	anthranilate phosphoribosyltransferase
17338_at	4,7	0,7	1,7	5,4E-03	2,1E-05	AT2G47550	pectinesterase family protein, contains Pfam profile: PF01095 pectinesterase
20429_at	8,5	-1,3	1,7	5,5E-04	1,5E-04	AT4G14400	ankyrin repeat family protein, contains ankyrin repeats, Pfam domain PF00023
13278_f_at	7,4	1,2	1,7	2,0E-03	3,6E-04	AT5G12030	17.7 kDa class II heat shock protein 17.6A (HSP17.7-CII)
14250_r_at	5,8	0,6	1,7	5,2E-02	4,3E-04	AT3G52430	phytoalexin-deficient 4 protein (PAD4)
20259_at	3,3	0,7	1,5	7,9E-04	9,3E-06	AT4G23200	protein kinase family protein, contains Pfam PF00069: Protein kinase domain
15647_s_at	5,9	0,4	1,4	1,1E-02	5,7E-06	AT4G14680	sulfate adenylyltransferase 3 / ATP-sulfurylase 3 (APS3)
15243_at	6,7	-0,5	1,4	1,1E-02	3,0E-05	AT4G39770	trehalose-6-phosphate phosphatase, putative, similar to trehalose-6-phosphate phosphatase (AtTPPB)

Table 3 A.

14781_at	6,6	-0,4	1,4	1,5E-02	8,7E-06	AT2G22850	bZIP transcription factor family protein
15859_at	7,1	0,7	1,3	1,6E-03	3,5E-05	AT2G28570	expressed protein
16140_s_at	5,9			1,9E-03	2,1E-04	AT1G21270	wall-associated kinase 2 (WAK2)
19463_s_at	7,3	1,0	1,3	2,0E-03	3,5E-04	AT4G39980	2-dehydro-3-deoxyphosphoheptonate aldolase 1 / 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 1 / DAHP synthetase 1 (DHS1)
18314_i_at	7,4	0,3	1,3	8,3E-02	7,2E-05	AT4G27830	glycosyl hydrolase family 1 protein, contains Pfam PF00232 : Glycosyl hydrolase family 1 domain;
19713_at	7,2	-0,8	1,2	5,3E-03	3,4E-04	AT4G18340	glycosyl hydrolase family 17 protein
16416_at	8,9	0,2	1,2	2,0E-01	2,2E-05	AT2G02130	plant defensin-fusion protein, putative (PDF2.3), plant defensin protein family member
17045_at	4,8	0,4	1,2	2,1E-02	4,3E-05	AT1G78090	trehalose-6-phosphate phosphatase (TPPB)
17119_s_at	5,8	0,5	1,2	2,2E-02	2,5E-04	AT2G06050	12-oxophytodienoate reductase (OPR3) / delayed dehiscence1 (DDE1)
13236_at	7,8	0,9	1,2	2,2E-03	4,0E-04	AT4G39980	2-dehydro-3-deoxyphosphoheptonate aldolase 1 / 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 1 / DAHP synthetase 1 (DHS1)
17464_at	8,2	0,7	1,1	5,1E-03	3,8E-04	AT1G09970	leucine-rich repeat transmembrane protein kinase
16963_at	7,2	0,4	1,1	3,1E-02	1,1E-04	AT2G38390	peroxidase, putative, similar to peroxidase isozyme (Armoracia rusticana)
16493_at	8,3	0,7	1,1	2,1E-03	1,2E-04	AT1G54010	myrosinase-associated protein, putative, similar to myrosinase-associated protein GI:1769969 from (Brassica napus)
12752_s_at	3,9	0,4	1,1	3,1E-02	1,6E-04	AT4G21960	peroxidase 42 (PER42) (P42) (PRXR1)
12744_at	6,8	0,1	1,0	2,8E-01	7,6E-05	AT3G16470	jacalin lectin family protein, contains Pfam profile: PF01419 jacalin-like lectin domain;
14697_g_at	4,7	0,2	1,0	2,2E-01	1,1E-04	AT2G21620	universal stress protein (USP) family protein / responsive to dessication protein (RD2)
17075_s_at	6,5	-0,2	1,0	2,6E-01	1,5E-04	AT5G22300	nitrilase 4 (NIT4)
12472_s_at	7,2	0,0	0,9	7,2E-01	1,5E-04	AT1G59960	aldo/keto reductase, putative, similar to NADPH-dependent codeinone reductase
15568_at	4,7	0,2	0,9	2,8E-01	5,4E-04	AT4G17910	zinc finger (C3HC4-type RING finger) family protein / pentatricopeptide (PPR) repeat-containing protein, contains Pfam domains
12202_at	5,1	0,5	0,9	1,1E-02	2,7E-04	AT3G50650	scarecrow-like transcription factor 7 (SCL7)
16902_at	6,9	0,2	0,9	1,1E-01	1,5E-04	AT2G21620	universal stress protein (USP) family protein / responsive to dessication protein (RD2),
16064_s_at	6,5	0,3	0,9	5,6E-02	2,6E-04	AT3G15210	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/AP2 transcription factor family (ATERF-4)
17513_s_at	7,3	0,5	0,9	7,9E-03	2,7E-04	AT1G26820	ribonuclease 3 (RNS3)
16610_s_at	6,5	0,1	0,8	5,5E-01	2,2E-04	AT1G19050	two-component responsive regulator / response regulator 7 (ARR7)
16061_s_at	7,1	0,5	0,8	1,3E-02	5,3E-04	AT4G26070	mitogen-activated protein kinase kinase (MAPKK) (MKK1) (MEK1)
16362_at	7,1	0,0	0,8	7,2E-01	1,9E-04	AT2G33050	leucine-rich repeat family protein, contains leucine rich-repeat domains Pfam:PF00560,
17641_g_at	5,2	0,2	0,8	1,9E-01	4,3E-04	AT4G10310	sodium transporter (HKT1)
17341_at	4,1	0,0	0,8	6,9E-01	3,1E-04	AT4G18250	receptor serine/threonine kinase, putative, similar to to receptor serine/threonine kinase PR5K
16643_s_at	4,5	0,2	0,8	2,0E-01	5,2E-04	AT3G45610	Dof-type zinc finger domain-containing protein
18626_at	5,9	-0,1	0,7	3,8E-01	3,4E-04	AT4G00780	meprin and TRAF homology domain-containing protein / MATH domain-containing protein, contains Pfam profile PF00917: MATH domain
13172_s_at	10,5	0,0	0,7	7,6E-01	5,0E-04	AT1G20840	transporter-related, low similarity to D-xylose proton-symporter (Lactobacillus brevis)

Genes involved in carbon assimilation and allocation, except those of T6P metabolism, are conspicuous by their absence in the tables with genes specifically regulated by trehalose (Tables 2&3). Exceptions to this observation are sucrose phosphatase 1, SPP1, and the glucose transporter STP1 which showed 2 and 3 fold down-regulation respectively by trehalose. This differs from feeding metabolisable sugars where expression of several enzymes of primary metabolism and transporters is strongly affected (Price et al., 2004; Blasing et al., 2005; Muller et al., 2007).

Table 3 B. Genes down-regulated by trehalose and unchanged by sucrose.

Probe set NA	A	Suc	Tre	p.value suc	p.value tre	Locus	description
16014_at	12,2	0,2	-2,0	3,6E-01	1,8E-05	AT1G75750	gibberellin-regulated protein 1 (GASA1) / gibberellin-responsive protein 1
16927_s_at	11,0	0,1	-1,1	3,3E-01	2,7E-05	AT4G30270	MERI-5 protein (MERI-5) (MERI5B) / endo-xyloglucan transferase / xyloglucan endo-1,4-beta-D-glucanase (SEN4)
12532_at	8,7	-0,4	-1,0	2,2E-02	1,2E-04	AT1G10760	starch excess protein (SEX1), identical to SEX1 (Arabidopsis thaliana)
17103_s_at	9,9	-0,6	-1,1	4,6E-03	1,5E-04	AT2G01850	xyloglucan:xyloglucosyl transferase / xyloglucan endotransglycosylase / endo-xyloglucan transferase (EXGT-A3)
19877_at	5,0	-0,7	-0,9	9,6E-04	3,2E-04	AT3G48390	MA3 domain-containing protein, similar to programmed cell death 4 protein (Gallus gallus)
18270_at	8,6	-0,3	-0,7	2,2E-02	3,3E-04	AT2G30930	expressed protein
20015_at	7,6	-0,8	-1,0	1,8E-03	3,9E-04	AT1G10020	expressed protein
20066_at	7,6	-0,5	-0,8	9,0E-03	5,2E-04	AT2G35840	sucrose-phosphatase 1 (SPP1), identical to sucrose-phosphatase (SPP1) (Arabidopsis thaliana)

Table 4. Genes typically affected by catabolic repression.

Probe set NA	A	suc	tre	p.value suc	p.value tre	Locus	description
16428_at	9,3	-5,4	-1,4	4,5E-08	3,9E-04	AT3G01500	carbonic anhydrase 1, chloroplast / carbonate dehydratase 1 (CA1
18087_s_at	9,6	-4,7	-0,6	3,1E-08	9,3E-03	AT1G03130	photosystem I reaction center subunit II, chloroplast, putative / photosystem I 20 kDa subunit, putative / PSI-D, putative (PSAD2)
16488_at	8,9	-4,3	-1,6	2,9E-08	2,2E-05	AT1G11260	glucose transporter (STP1), nearly identical to glucose transporter GB:P23586 SP:P23586 from (Arabidopsis thaliana)
15144_s_at	8,6	-4,3	-1,3	1,8E-07	4,0E-04	AT5G14740	carbonic anhydrase 2 / carbonate dehydratase 2 (CA2) (CA18)
18276_at	10,0	-4,2	-0,6	2,2E-07	1,9E-02	AT1G03600	Photosystem II family protein, similar to SP:P74367 {Synechocystis sp.}
11994_at	5,7	-4,0	-1,1	1,4E-06	3,7E-03	AT2G39470	photosystem II reaction center PsbP family protein
16034_at	9,4	-3,7	-0,8	2,2E-07	2,7E-03	AT1G32060	phosphoribulokinase (PRK) / phosphopentokinase,
13678_at	10,1	-3,7	-1,0	1,4E-07	7,5E-04	AT1G19150	chlorophyll A-B binding protein, putative / LHCI type II, putative, very strong similarity to PSI type II chlorophyll a/b-binding protein Lhca2*1 GI:541565
16004_s_at	11,4	-3,6	-0,1	2,0E-07	6,7E-01	AT3G54890	chlorophyll A-B binding protein / LHCI type I (CAB), identical to chlorophyll A/B-binding protein (Arabidopsis thaliana)
16673_at	8,5	-3,6	-1,0	1,1E-07	5,7E-04	AT5G23120	photosystem II stability/assembly factor, chloroplast (HCF136
16485_s_at	10,1	-3,5	-0,8	4,7E-07	4,9E-03	AT3G50820	Encodes a protein which is an extrinsic subunit of photosystem II and which has been proposed to play a central role in stabilization of the catalytic manganese cluster.
16899_at	9,6	-3,5	-0,5	6,5E-07	4,2E-02	AT1G51400	photosystem II 5 kD protein, 100% identical to GI:4836947 (F5D21.10)
13213_s_at	11,6	-3,5	0,1	6,9E-07	6,8E-01	AT3G54890	chlorophyll A-B binding protein / LHCI type I (CAB
15133_at	11,8	-3,4	-0,2	4,0E-08	2,9E-01	AT3G61470	chlorophyll A-B binding protein (LHCA2), identical to Lhca2 protein (Arabidopsis thaliana) GI:4741940
16497_at	10,4	-3,4	-0,2	4,4E-07	2,5E-01	AT3G21055	photosystem II 5 kD protein, putative
17087_at	10,4	-3,4	-0,5	1,5E-06	7,0E-02	AT5G64040	photosystem I reaction center subunit PSI-N, chloroplast, putative / PSI-N, putative (PSAN), SP:P49107
15154_at	8,3	-3,3	-1,8	1,1E-05	4,8E-04	AT3G47340	asparagine synthetase 1 (glutamine-hydrolyzing) / glutamine-dependent asparagine synthetase 1 (ASN1)
15153_at	7,1	-3,2	-1,5	1,0E-04	9,5E-03	AT3G27690	chlorophyll A-B binding protein (LHCB2:4)
12610_at	7,5	-3,2	-0,7	3,1E-06	2,2E-02	AT3G51820	chlorophyll synthetase, putative
16449_s_at	11,8	-3,1	-0,2	2,5E-07	1,6E-01	AT5G66570	Encodes a protein which is an extrinsic subunit of photosystem II and which has been proposed to play a central role in stabilization of the catalytic manganese cluster
16018_s_at	9,4	-3,1	-0,6	4,9E-07	1,1E-02	AT3G26650	glyceraldehyde 3-phosphate dehydrogenase A, chloroplast (GAPA) / NADP-dependent glyceraldehydephosphate dehydrogenase subunit A
16424_g_at	11,5	-3,0	-0,4	4,9E-07	5,8E-02	AT2G30570	photosystem II reaction center W (PsbW) protein-related
18077_at	10,8	-3,0	-0,2	5,8E-07	2,4E-01	AT1G31330	photosystem I reaction center subunit III family protein, contains Pfam profile: PF02507: photosystem I reaction center subunit III
12611_g_at	7,6	-3,0	-0,7	1,7E-06	8,9E-03	AT3G51820	chlorophyll synthetase, putative
18081_at	10,4	-3,0	-0,4	3,1E-07	2,7E-02	AT1G52230	photosystem I reaction center subunit VI, chloroplast, putative / PSI-H, putative (PSAH2)

Table 4.

17411_s_at	7,0	-3,0	-0,8	4,1E-07	2,0E-03	AT1G77490	L-ascorbate peroxidase, thylakoid-bound (tAPX), identical to thylakoid-bound ascorbate peroxidase GB:CAA67426 (Arabidopsis thaliana)
18082_at	11,6	-2,8	-0,3	5,8E-07	7,0E-02	AT1G30380	photosystem I reaction center subunit psaK, chloroplast, putative / photosystem I subunit X, putative / PSI-K, putative (PSAK)
15102_s_at	11,4	-2,8	-0,2	2,9E-07	3,4E-01	AT4G10340	chlorophyll A-B binding protein CP26, chloroplast / light-harvesting complex II protein 5 / LHCIIc (LHCB5)
15182_at	10,2	-2,8	-0,9	8,2E-07	9,5E-04	AT1G68010	glycerate dehydrogenase / NADH-dependent hydroxypyruvate reductase
18088_i_at	10,7	-2,8	-0,2	7,2E-07	3,0E-01	AT4G28750	photosystem I reaction center subunit IV, chloroplast, putative / PSI-E, putative (PSAE1)
14729_s_at	11,4	-2,7	-0,5	3,9E-07	9,5E-03	AT3G26650	glyceraldehyde 3-phosphate dehydrogenase A, chloroplast (GAPA) / NADP-dependent glyceraldehydephosphate dehydrogenase subunit A
18084_s_at	11,8	-2,6	-0,3	4,8E-07	1,1E-01	AT4G12800	photosystem I reaction center subunit XI, chloroplast (PSI-L) / PSI subunit V,
13234_s_at	11,0	-2,6	-0,2	7,9E-07	1,9E-01	AT1G20340	plastocyanin, similar to plastocyanin GI:1865683 from (Arabidopsis thaliana)
15097_at	11,9	-2,6	-0,3	3,2E-07	8,0E-02	AT1G15820	chlorophyll A-B binding protein, chloroplast (LHCB6)
18665_r_at	5,5	-2,6	-0,4	3,2E-05	1,6E-01	AT2G20260	photosystem I reaction center subunit IV, chloroplast, putative / PSI-E, putative (PSAE2)
18666_s_at	10,3	-2,6	-0,2	5,8E-06	2,9E-01	AT2G20260	photosystem I reaction center subunit IV, chloroplast, putative / PSI-E, putative (PSAE2)
15995_at	12,2	-2,6	-0,1	5,3E-07	4,0E-01	AT5G01530	chlorophyll A-B binding protein CP29 (LHCB4)
15373_g_at	9,2	-2,5	-0,8	1,1E-05	1,3E-02	AT1G76100	plastocyanin, identical to plastocyanin GI:1865683 from (Arabidopsis thaliana)
16423_at	11,8	-2,5	-0,3	3,2E-07	7,7E-02	AT2G30570	photosystem II reaction center W (PsbW) protein-related
18086_s_at	10,5	-2,5	-0,3	3,3E-06	1,6E-01	AT4G02770	photosystem I reaction center subunit II, chloroplast, putative / photosystem I 20 kDa subunit, putative / PSI-D, putative (PSAD1)
16417_s_at	11,9	-2,5	-0,1	1,6E-06	4,5E-01	AT4G12800	photosystem I reaction center subunit XI, chloroplast (PSI-L) / PSI subunit V,

This may imply that control of carbon assimilation for growth by accumulating T6P is mainly mediated at the post-transcriptional level. A previous report revealed that sucrose starvation involves substantial translational repression: most of the mRNA appears to be translationaly repressed (183/245 genes) when starving Arabidopsis cells from sucrose (Nicolai et al., 2006). In conclusion, micro-array profiling allows characterizing the physiological state of seedlings after 24 h on trehalose but it does not allow distinguishing reactions due to T6P or to trehalose. T6P steady state concentrations in plants with altered T6P metabolism were combined with microarray profiling to identify a cluster of genes that responds to T6P concentrations (Schluepmann et al., 2004) and this identified some putative targets of T6P. To reveal components of T6P control over growth and allocation, identifying mutants impaired in T6P responses is a most interesting alternative approach.

Reference

Benjamini, Y., and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Jornal of the Royal Statistical 57, 300-305.

Blasting, O.E. Gibson, Y., Gunther, M., Hohne, M., Morcuende, R., Osuna, D., Thimm, O., Usadel, B., Scheible, W.R., and Stitt, M., 2005. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. Plant Cell 17, 3257-3281.

Crowe, J.H., Hoekstra, F.A., and Crowe, L.M., 1992. Anhydrobiosis. Annu. Rev. Physiol. 54, 579-599.

Elbein, A.D., 1974. The mechanism of alpha, alpha trehalose. Adv. Carbohydr. Chem. Biochem. 30, 227-252.

Elbein, A.D., Pan, Pastuszak, Y.T., and Carroll, D., 2003. New insights on trehalose: a multifunctional molecule. Glybilogy 13, 17R-27R.

Freudenberg, J., Boriss, H., and Hasenclever, D., 2004. Comparison of preprocessing procedures for oligonucleotide micro-array by parametric bootstrap simulation of spike-in experiments. Methods Inf. Med. 43, 434- 438.

Fritzius, T., Aeschbacher, R., Wiemken, A., and Wingler, A., 2001. Induction of ApL3 expression by trehalose complements the starch-deficient Arabidopsis mutant *adg2-1* lacking ApL1, the large subunit of ADP-glucose pyrophosphorylase. Plant physio. 126, 883-889.

Goddijn, O.J., Verwoerd, T.C., Voogd, E., Krutwagen, R.W., De Graaf, P.T., Van Dun, K., Poels, J., Ponstein, A.S., Damm, B., Pen, J., 1997. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol. 113, 181-190.

Gutierrez, R.A., Lejay, L.V., Dean, A., Chiaromonte, F., Shasha, D.E., Coruzzi, G.M., 2007. Qualitative network models and genome-wide expression data carbon/nitrogen-responsive molecular machines in Arabidopsis. Genome Biol. 8, R7.

Huber, W., Von Heydebreck, A., Sultmann, H., Poustka, A., Vingron, M., 2002. Variance stabilization applied to microarray data calibration to the quantification of differential expression. Bioinformatics 18, Supp 1. 96-104.

Kolbe, A., Tissen, A., Schluepmann, H., Paul, M., Ulrich, S., Geigenberger, P., 2005. Trehalose-6-phosphate regulates starch synthesis via posttranslation redox activation of ADP-glucose pyrophosphorylase. Proc. Natl. Acad. Sci. USA. 102, 11118-11123.

Lim, JD., Cho, JI., Park, YI., Hahn, TR., Choi, SB., Joen, JS., 2006. Sucrose transport from source to sink seeds in rice. Physiology Plantarum 126, 572-584.

Loeffler, C., Berger, S., Guy, A., Durand, T., Bringmann, G., Dreyer, M., Von Rad, U., Durner, J., Muller, M.J., 2005. B1-phytoprostanes trigger plant defense and detoxification responses. Plant Physiol. 137, 328-340.

Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., Sheen, J., 2003. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light and hormonal signaling. Science 300, 332-336.

Muller, J., Boller, T., Wiemsken, A., 1995. Trehalose and trehalase in plants: recent developments. Plant science 112, 9-15.

Muller, R., Morant, M., Jarmer, H., Nilsson, L., Nielsen, T.H., 2007. Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. Plant Physiol. 143, 156-171.

Nawrath, C., Heck, S., Parinthawong, N., Metraux, J.P., 2002. EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. Plant cell 14, 275-286.

Nicolai, M., Roncato, M.A., Canoy, A.S., Rouquie, D., Sarda, X., Freyssinet, G., Robaglia, C., 2006. Large-scale analysis of mRNA translation states during sucrose starvation in Arabidopsis cells identifies cell proliferation and chromatin structure as targets of translational control. Plant Physiol. 141, 663-673.

Pellny, T.K., Ghannoum, O., Conroy, J.P., Schluepmann, H., Smeekens, S., Andralojc, J., Krause, K.P., Goddijn, O., Paul, M.J., 2004. Genetics modification of photosynthesis with E.Coli genes for trehalose synthesis. Plant Biotechnol. J. 2, 71-82.

Price, J., laxmi, A., St Martin, S.K., Jang, J.C., 2004. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. Plant Cell 16, 2128-2150.

Ramon, M., Rolland, F., Thevelein, J.M., Van Dijck, P., Leyman, B., 2007. ABI4 mediates the effects of exogenous trehalose on Arabidopsis growth and starch breakdown. Plant Mol. Biol. 63, 195-206.

Schluepmann, H., Van Dijken, A., Aghdasi, M., Wobbes, B., Paul, M., Smeekens, S., 2004. Trehalose mediated growth inhibition of Arabidopsis seedlings is due to trehalose-6-phosphate accumulation. Plant Physiol. 135, 879-890.

Smyth, G.K., 2004. Linear models and empirical bayes methods fro assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, 1-26.

Van Dijken, A., Schluepmann, H., Smeekens, S., 2004. Arabidopsis trehalose-6-phosphate synthase1 is essential for normal vegetative growth and transition to flowering. Plant Physiol. 135, 969-977.

Wingler, A., Fritzius, T., Wiemken, A., Boller, T., Aeschbacher, R.A., 2000. Trehalose induces the ADP-glucose pyophosphorylase gene, APL3, and starch synthesis in Arabidopsis. Plant Physiol. 124, 105-114.