Cloning and expression analysis of two photosynthetic genes, *PSI-H* and *LHCB1,* under trehalose feeding conditions in *Arabidipsis* seedlings

Mahnaz Aghdasi[1,2*], Henriette Schluepman[2]

[1]Departement of Biology, Faculty of Science, Golestan University, Gorgan, P.O. Box 155, I.R. Iran [2]Department of Molecular Plant Physiology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Abstract
Trehalose (α-D-glucosyl-[1,1]-α-D-glucopyranoside) is involved in mechanisms that coordinate metabolism with plant growth adaptation and development. The main objective of the current work was to find out whether trehalose feeding affects the expression of two genes involved in photosynthesis: one gene coding for photosystem1 subunit H (*PS1-H)* and the other for the light harvesting complex B1 (*LHCB1*). In this study, *Arabidopsis* seeds were grown on one-half-strength medium supplemented with 100 mM trehalose or sorbitol (as osmotic control) for 2 weeks. Trehalose-fed seedlings showed inhibited root growth, delayed emergence of primary cotyledons and dark-rimmed cotyledons. Exogenously applied trehalose strongly induced starch accumulation in cotyledons and, concomitantly, depletion of starch in collumella cells of the root cap. Gene expression analysis of photosynthetic genes in *Arabidopsis* seedlings revealed that trehalose feeding repressed *PS1-H* and *LHCB1* expression as compared to sorbitol-fed seedlings. To confirm trehalose inhibitory effect on photosynthetic gene expression, the cDNA of *PS1-H* and *LHCB1* was used to transform *Arabidopsis* seedlings. Transformed lines showed higher transcript levels of *PS1-H* and *LHCB1.*Trehalose feeding also reduced expression levels of *PS1-H* and *LHCB1* in transformed lines. These findings show that trehalose down-regulates the expression of two genes encoding typical components of the photosynthetic machinery.
Keywords: *PS1-H*; *LHCB1*; gene expression; transformation; *Arabidopsis*

Correspondence to:* ***Mahnaz Aghdasi,* Ph.D.
Tel: +98 171 4427173; Fax: 0171 4427040
E-mail: Aghdasi46@yahoo.com

INTRODUCTION

Environmental variables such as light, temperature and nutrition affect the rate of carbon assimilation in photosynthesis. Photosynthesis is also regulated by carbohydrate end-products. Accumulation of sugars like glucose and sucrose typically down regulates the expression of a number of genes encoding some components of the photosynthetic machinery, such as *LHCB1* (Vinit *et al*., 2005).

Sugars, irrespective of their roles as a rich sources of carbon and energy have an important signaling role in plant growth and development. They can regulate cell division, growth, differentiation, metabolism, and resource allocation in plants (Smeekens, 2000; Koch, 1996). The elaboration of the photosynthesis machinery during plant development in some tissues or organs like foliage leaves converts them to an active source of carbohydrate production. The carbohydrates thus produced can be exported from source to sink tissues like roots, fruits or tubers where they can be utilized for growth or storage (Noctor and Foyer, 2000). Therefore, the expression of enzymes involved in carbohydrate production in source tissues must be integrated with its utilization in sink tissues (Koch, 1996).

The most striking example of an altered carbohydrate allocation is that observed when *Arabidopsis* seedlings were grown on a trehalose containing medium (Wingler *et al*., 2000). When supplied to the medium, trehalose spurs the massive accumulation of starch in the source tissue whereas traditional sinks like

meristems and roots remain carbon starved and thus growth is inhibited. Trehalose (α-D-glucosyl-[1,1]-α-D-glucopyranoside) is a non-reducing disaccharide which consists of two glucose units joined by an α-1, 1 linkage. Trehalose is a common sugar in nature (Elbein, 1974) and present in a wide variety of organisms (Elbein *et al*., 2003). Plants generally contain only trace amounts of trehalose (Zentella *et al*., 1999; Muller *et al*., 1995). Exceptions to this exist and these are plants with extreme drought stress resistance such as *Selaginella lepidophylla* that accumulate quantitative amounts of trehalose (Zentella *et al*., 1999). All plants seem to contain genes for trehalose metabolism (Shima *et al*., 2007; Leyman *et al*., 2001; Blazquez *et al*., 1998; Vogel *et al*., 1998). Synthesis of trehalose-6-phosphate (T6P), the immediate precursor of trehalose, is indispensable for development of the *Arabidopsis* embryo (Eastmond *et al*., 2002). Evidence is thus accumulating that suggests T6P has an important regulatory role in carbon utilization of *Arabidopsis* seedlings (Schluepmann *et al*., 2003). Tobacco plants engineered with the *Escherichia coli* trehalose-6-phosphate synthase and trehalose phosphate phosphatase (*TPS* and *TPP*) genes are altered in their T6P steady state and exhibit the pleiotropic phenotypes. Plants with high T6P have smaller and darker green leaves, while plants with low T6P have larger pale green leaves (Pellny *et al*., 2004). How T6P controls carbon utilization and why changes in the steady state level of this metabolizing signaling molecule yield such strong phenotypic changes require further investigations.

Production of high energy reducing equivalents during photosynthesis of higher plants requires the coordinated participation of two different photosynthetic systems, i.e., photosystems I (PSI) and II (PSII). PSI is a multi-protein complex consisting of 17 different subunits. Subunit H (PSI-H) is specially important for state transitions in plant photosynthesis. In plants lacking PSI-H, transfer of energy to PSI through the light harvesting complex (LHCII) would be impaired (Lunde *et al*., 2000). The light harvesting complex of PSII i.e. LHCII contains more than 60% of all kinds of chlorophyll in plants. These antenna complexes absorb sunlight and transfer excitation energy to the core complex of photosystem II (PSII) reaction center. LHCII consists of three proteins which are encoded by *LHCB1, LHCB2* and *LHCB3* genes. *LHCB1* and *LHCB2* are the most abundant proteins in the LHCII complex (Ruban *et al*., 1994).

So far, there is not any report describing the effects of trehalose on the expression of genes encoding components of photosynthesis. In this study, we investigated whether exogenous trehalose has any effect on the expression of photosynthetic genes (*PSI-H* and *LHCB1).*To achieve this goal, *Arabidopsis* plants over expressing two photosynthetic genes were produced and the effects of exogenous trehalose on the expression of these genes were compared between wild type and transformed plants.

MATERIALS AND METHODS

Plant materials and growth conditions: The *Arabidopsis thaliana* wild type (WT) plants accession Columbia-0 (COL-0) was used in this study. Seeds were surface sterilized for 5 min with 70% (v/v) ethanol followed by 10 min in 20% commercial bleach (4% (w/v) chlorine) and washed 5 times in sterile milli-Q water. Sterilized seeds were plated on half-strength Murashige and Skoog medium (1962) (MS) supplemented with either 100 mM trehalose or sorbitol and solidified with 0.8% (w/v) agar.

Seeds were stratified in darkness at 4ºC for 2 days before the plates were transferred to a growth chamber at 25ºC under a 16-h-light/8-h-dark photoperiod. Seedlings were grown vertically for 14 days. After 14 days, seedlings were photographed and root length was determined using the Image J program (Wayne Rasband, NIH Maryland, USA).

Starch staining and measurement: For analysis of starch distribution, whole seedlings were taken and destained in 70% and then 90% (v/v) ethanol. Starch staining of seedlings was carried out by using the KI/I_2 solution, followed by washing milli Q water. Pictures were taken by the Normanski microscope (Zeiss, Jena, Germany).

To determine starch levels, 50 mg of fresh weight plant material (whole seedlings) was frozen and grounded in liquid nitrogen. Sugars were extracted with 1 ml of 80% (v/v) ethanol for 10 min at 80ºC in Eppendorf tubes. The tubes were spun for 5 min at 13000 rpm and the supernatant was transferred to a clean tube. The pellet was extracted again with 75 µl of milliQ water for 10 min at 80ºC and then centrifuged at 23000 ×*g* the supernatants were transferred

to a clean tube. The resulting supernatants were then pooled. Starch was then extracted from the remaining pellets by incubation in 0.1 ml of 0.5 M NaOH at 60ºC for 30 min. After addition of 6 µl of acetic acid (96%), starch was digested overnight at 37ºC by addition of amyloglucosidase. Starch content was measured by hexokinase activity (Boehringer Mannheim, Darmstadt, Germany) as described by the manufacturer.

Chlorophyll and anthocyanin measurements: Chlorophylls a, b and total chlorophyll were determined spectrophotometrically as described by Jeffery and Humphery (1975). In brief, 14 days seedlings were grounded in liquid nitrogen and extracted with 80% (v/v) acetone. Absorbance was then measured at 647, 652 and 664nm. Concentrations of chlorophylls a, b and total chlorophyll were calculated by using extinction coefficients.

Anthocyanin content of seedlings was determined using the protocol of Mita *et al*. (1967). Frozen and homogenized seedlings (20 mg) were extracted for 1 day at 4°C in 1 ml of 1% (v/v) hydrochloric acid in methanol. The mixture was centrifuged at 23,000 ×*g* for 15 minutes and the absorbance of the supernatant was measured at 530 and 657 nm. Relative anthocyanin concentrations were calculated using the formula [*A*530-(1/4 × *A*657)]. The relative anthocyanin content was defined as the product of relative anthocyanin concentration and the extract volume. One anthocyanin unit equals to one absorbance unit [*A*530-(1/4 × *A*657)] in 1 ml of the extraction solution.

RNA extraction and cDNA synthesis: Total RNA was extracted from 10 days old *Arabidopsis* plants. Whole plant material was snap frozen in liquid nitrogen, pulverized with glass beads for 2 minutes at 2800 rpm in a dismembrator (Braun, Melsungen, Germany). Total RNA was isolated with the RNeasy plant mini kit (QIAGEN USA, Valencia, CA). RNA concentration and purity were determined by measuring the absorbance at 260 nm. To remove any possible contamination by genomic DNA, 10 ng of RNA was treated with 2 U of DNAse I (DNA- free, Ambion, Austin, USA). The absence of DNA was analyzed by performing a PCR reaction (40 cycles, similar to the real–time PCR program) on the DNaseI-treated RNA using *Taq* DNA polymerase. Reverse transcriptase PCR (RT-PCR) experiments were performed using 1 ng of total extracted RNA and used for first-strand cDNA synthesis with 60 U of M-MLV reverse transcriptase (Promega, Madison, WI), 0.5 µg of odT16v (custom oligo from Invitrogen, Carlsbad, CA) and 0.5 µg of random hexamer (Invitrogen, USA). PCR was performed with reverse and forward primers (*PSI-H*: 5′-tacacaaaattcccactcacca-3′ and 5′-gggtttgcagtattgagaaaat-3′, *LHCB1*: 5′-ctcaacaatggctctctcct-3′ and 5′-aacccaagaactgaaaatccaa-3′). Amplification conditions were performed as initial DNA denaturation at 94ºC for 2 minutes followed by 35 cycles of 1 minutes denaturation at 94ºC, 30 second annealing at 56ºC and 2 minutes of extension at 72ºC with a final extension time at 72ºC for 10 minutes. An aliquot of the PCR product was run on an agarose gel (1%) and the remaining PCR product was cleaned using a DNA purification kit (Amersham Biosciences, England).

Cloning cDNA fragments into pGEM-T Easy vector: The resulting cDNA fragments from the previous step were ligated into the pGEM-T Easy vector. For this purpose, cDNA was concentrated to 3 µl (25 ng) and was then added to 5 µl of 2 × ligation buffer, 1 µl of T4 Ligase and 1 µl of pGEM-T easy vector. The ligation mixture was incubated over night at room temperature.

An aliquot (100 µl) from the competent *E. coli* were taken from the -80ºC freezer and thawed on ice for 20 min. The over night ligation mixture was added to the cells. The mixture was left on the ice for 20 minutes. Heat shock was applied for 50 sec at 42ºC, followed by a 5 min cooling period on ice. One ml of lysogeny broth (LB) medium was added and cells were incubated at 37ºC for 1 h. The LB plates contained 50 µg/ml of ampicillin for selection. Isopropyl-β-D-1-Thiogalactopyranoside (IPTG) and X-Galactopyranoside (X-Gal) were added for screening of blue and white colonies. To check colonies containing the plasmid with the ligated fragment, restriction enzyme analysis was performed. Plasmids were isolated from 5 colonies using a plasmid miniprep kit (Sigma, USA). In the digestion mixture, 2 µl of plasmid, 1 µl of 10 X buffer, 6 µl of milli-Q water and 1 µl of *Eco*R1 were used. Samples were digested at 37ºC for 1.5 h. The obtained fragments were analyzed by agarose gel electrophoresis.

Sequence analysis: DNA sequencing was carried out at the sequencing facility in Wageningen University.

Table 1. Q-PCR primer sets used in this study. Eff means primer's efficiency for each set of primers. Primer efficiencies for all primer sets were determined according to equation E=-1/slope, as described by Rasmussen (2000).

Gene	Locus	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Efficiency
PSI-H	At3g16140.1	cccacttcagagcaagttcttt	cccacttcagagcaagttcttt	1.87
LHCB1	At2g29920	ggggtcagcggatagaccag	ctttcgccggaaaggctgt	1.80
AtTRE1	At4g24040	gctgcaccacgaaccagtaga	ttcttcgttctccacgttgga	1.98

Sequences obtained from analysis with forward and reverse primers (T7: 5′ tatttaggtgacactatag 3′ and SP6: 5′ taatacgactcactataggg 3′) were aligned and the PCR fragment structures reconstructed by BLAST (Basic Local Alignement Search Tool) searches in TAIR (http://www.arabidopsis.org/Blast/).

cDNA over-expression constructs and re-transformation into Col-0: Full length cDNA were isolated and purified from the pGEM-T easy vector clones and over-expressed in wild type (WT) *Arabidopsis* plants. The CaMV 35S expression cassette was isolated by digestion with *Eco*RV from the pUC-18 vector. The cassette was filled with Klenow and dNTP and subsequently ligated into pBin19 (*Hin*dIII/*Eco*RI) to yield pBin-35S. Purified fragments were cloned into the pBin-35S expression cassette, resulting in pBin35S/cDNA/NOS. The plasmids containing photosynthetic genes were digested with *Xba*I and *Xmn*I restriction enzymes. The construct was introduced by electroporation into *Agrobacterium tumefaciens* containing the pGV2260 plasmid. The resulting bacteria were used to transform *Arabidopsis* by floral dip method (Clough and Bent, 1998). Transgenic seedlings were selected on half MS media containing 50 mg/l of Kanamycin. Transgenic seedlings were grown in soil medium under long-day conditions (16-hlight/ 8-h-dark) for further growth and characterization.

Expression analysis of the photosynthetic genes: Quantitative-PCR (Q-PCR) analysis was performed to determine the expression level of *LHCB1* and *PSI-H* photosynthetic genes. Total RNA was extracted from *Arabidopsis* seedlings as mentioned above. Following treatment of RNA with DNAase I, cDNA was synthesized using the M-MLV reverse transcriptase system (Promega, Madison, WI). Q-PCR was carried out by ABI-prism 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA). For each reaction, 12.5 µl of green PCR Master Mix (Applied Biosystems, UK) and 2.5 µl of gene-specific primers were used (Table1). Each experiment was repeated 3 times. Relative quantitation of gene expression is based on the comparative Ct method (User Bulletin No. 2: ABI PRISM 7700 sequence detection system, 1997) using *AtACTIN2* as the calibrator reference (f: 5′-ATGTCTCTTACAATTTCCCG-3′ and r: 5′-CCAACAGAGA-GAAGATGACT-3′). The Q-PCR data were normalized against *AtACTIN2*. The results are expressed as a target/reference ratio.

Trehalase gene expression analysis: To find out if trehalose is sensed by transformed lines and WT seedlings growing on 100 mM trehalose, a Q-PCR analysis was performed by *AtTRE1*-specific primers (Table 1). The expression level of the only trehalase gene (*AtTRE1*) was analyzed in both WT and transformed lines.

RESULTS

Trehalose effects on plant growth and development: *Arabidopsis thaliana* (WT) plants were fed with trehalose (100 mM) for two weeks, showed inhibited root growth, delayed emergence of priming leaves and dark red-rimmed cotyledons (Fig. 1A). Trehalose feeding induced starch accumulation in cotyledons and depletion of starch in the columella cells of the root cap (Fig. 1 B-C). However, WT seedlings growing on 100 mM sorbitol contained 11 mg/g of fresh weight (FW) starch. Ttrehalose feeding led to the accumulation of starch up to 52 mg/g of FW (Fig. 1 D).

Chlorophyll and anthocyanin contents were also determined in both trehalose and sorbitol-fed plants. The total chlorophyll contents of WT seedlings after 14 days of growth on trehalose was 4-fold less than that on sorbitol (Fig. 2A). The Chla/Chlb ratio was increased from 2.13 in sorbitol-fed plants to 3.75 in

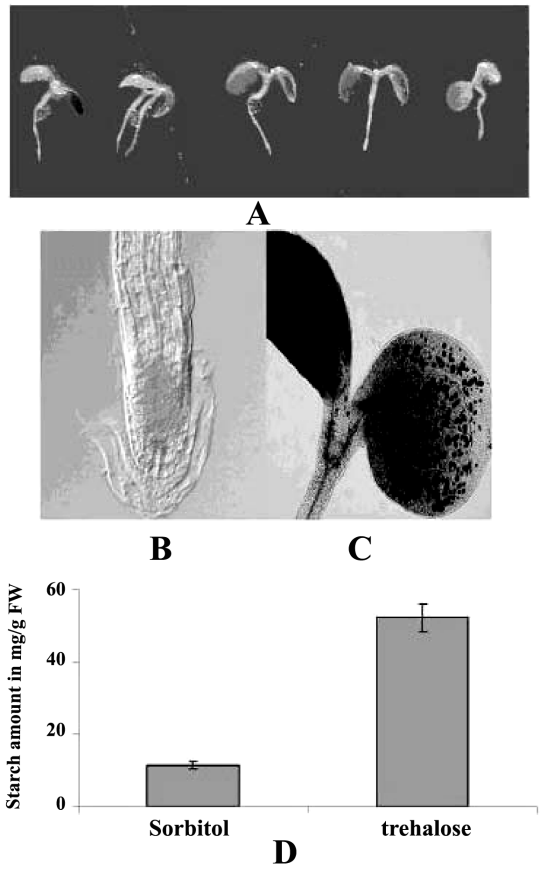

Figure 1. Description of the growth arrest, starch staining and quantification in WT seedlings growing on 100 mM trehalose. A: WT seedlings grown on 100 mM trehalose after 14 days under long-day conditions, B: Typical starch staining in the root tips of WT, C: Starch accumulation in cotyledons, d: Starch quantifications in whole seedlings. WT: Wild Type, FW: fresh weight.

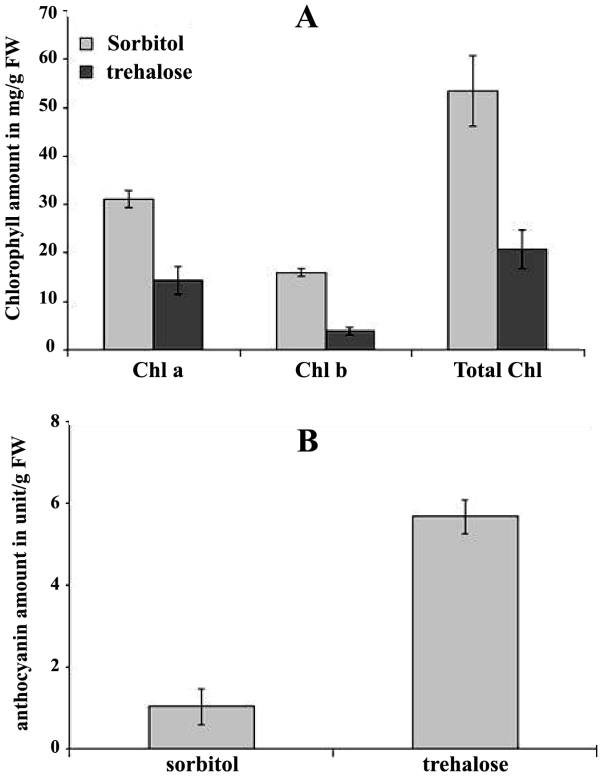

Figure 2. The effect of 100 mM trehalose feeding on chlorophyl and anthocyanin levels in WT seedlings. Seedlings were grown for 14 days on either 100 mM sorbitol or trehalose under long-day conditions. A: chlorophyll and B: anthocyanin contents. WT: Wild Type, Chla: chlorophyll A, Chlb: chlorophyll B, Total Chl: total chlorophyll, FW: fresh weight.

trehalose-fed ones. Anthocyanin was 5-fold higher in WT seedlings after 14 days of growth on trehalose, when compared to sorbitol (Fig. 2B).

Expression analysis of photosynthetic genes in *Arabidopsis* seedlings growing on 100 mM trehalose opposed to sorbitol: By using Q-PCR, expression levels of two photosynthetic genes *PSI-H* and *LHCB1* were determined in both10 days old trehalose and sorbitol-fed seedlings grown on half-strength MS medium. Levels of gene expression were determined with reference to *AtACTIN2*. Trehalose feeding repressed *PSI-H* and *LHCB1* expression as compared to the control plants (Fig. 3).

Transformation of cDNA constructs into WT: Transformations with the full length cDNAs of *PSI-H* and *LHCB1* yielded 20 independent lines per construct with resistance to the selection marker. In soil and under long-day conditions, *PSI-H* transformed plants displayed somewhat larger green leaves compared to WT non-transformed ones (Fig. 4). Plants which over expressed *LHCB1* had dark green leaves when compared to WT plants (data not shown). The transformed lines remained unaltered with respect to flowering time and were fully fertile.

Transformation yielded plants with higher expression levels of *PSI-H* and *LHCB1*. The mRNA levels of *PSI-H* in transformed lines were nearly 2 times higher than that of WT plants (Fig. 5A). The transcript level of *LHCB1* in transformed lines was significantly higher than that of WT plants (Fig. 5B).

Expression analysis of the transformed lines on 100 mM trehalose: The expression levels of endogenous genes homologous to the cDNA transgenes were fur-

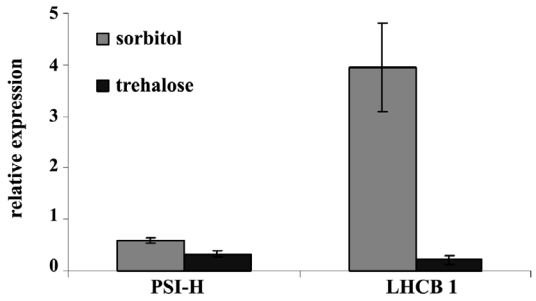

Figure 3. Q-PCR results of *PSI-H* and *LHCB1* expression in WT seedlings grown on half-strength MS supplemented with 100 mM sorbitol or trehalose.

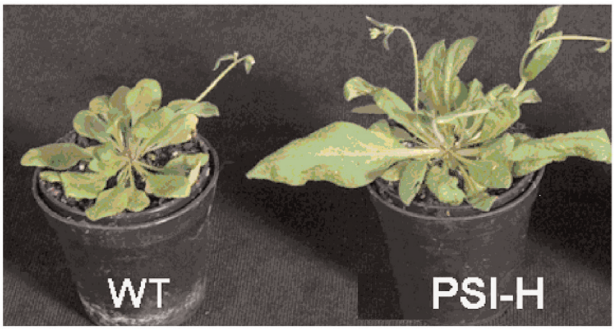

Figure 4. Phenotypes of the *PSI-H* transformed lines, as compared to the WT. Plants were grown in soil at 22°C under long-day conditions.

ther analyzed in 10 days old transformed seedling lines growing on 100 mM sorbitol or trehalose. The expression of *PSI-H* in trehalose-fed transformed lines was two times less than that in sorbitol-fed plants. Trehalose feeding also reduced the expression of *LHCB1* by 8-fold in the transformed lines (Fig. 6).

Trehalase expression: Trehalase expression analysis was performed using mRNA from 10 days old

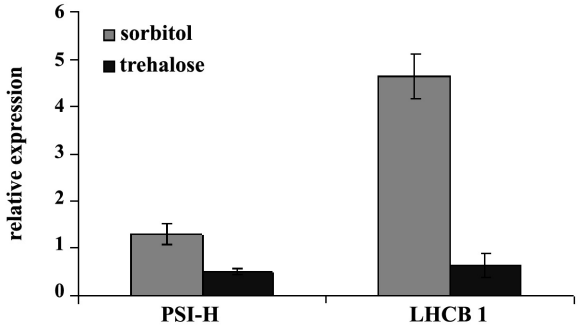

Figure 6. Relative *PSI-H* and *LHCB1* expression by Q-PCR in transformed lines growing on 100 mM sorbitol or trehalose.

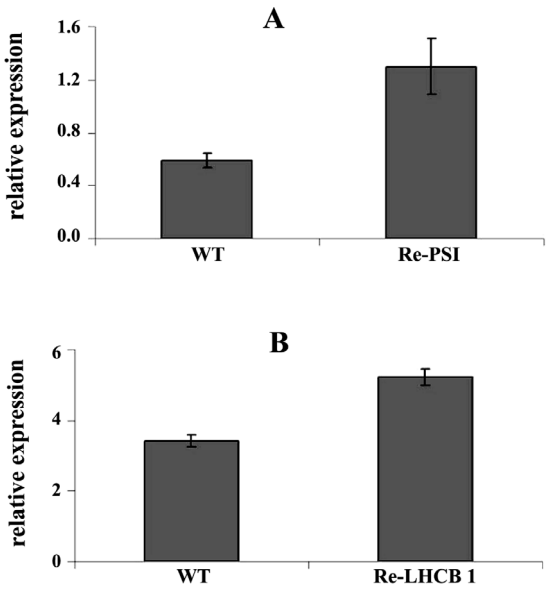

Figure 5. Expression levels of *PSI-H* and *LHCB1* encoded by the cDNA of transformed lines. Expression levels of A: *PSI-H* and B: *LHCB1* genes in transformed lines, as compared to WT.

seedlings grown on half-strength MS medium with 100 mM sorbitol or trehalose. Trehalose feeding induced trehalase expression in WT and transformed lines. WT seedlings growing on trehalose showed a 7-fold increase in the expression of *AtTRE1* when compared to those growing on sorbitol. *AtTRE1* expression in seedlings of the transformed lines growing on trehalose was similarly induced when compared to WT plants (Fig. 7). All transformed lines displayed comparable levels of trehalase expression when cultured under similar conditions.

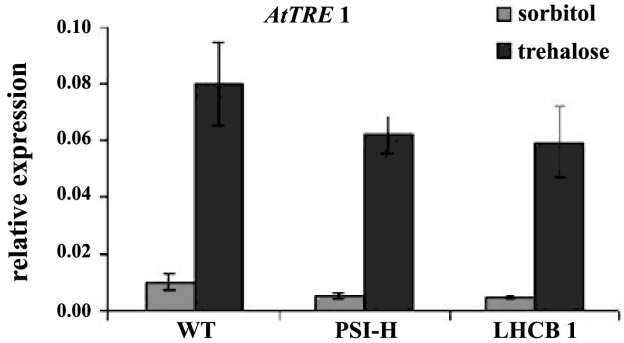

Figure 7. *AtTRE1* expression level in *PSI-H* and *LHCB1* transformed lines, as compared to WT grown on 100 mM sorbitol or trehalose. PSI-H: *PSI-H* transformed lines, LHCB1: *LHCB1* transformed lines.

DISCUSSION

Trehalose metabolism is emerging as an important new regulator of plant growth, metabolism, and stress resistance. There are evidences, which show that minor alterations in trehalose metabolism yield dramatic and pleiotropic phenotypic changes in plants (Ramon *et al*., 2007; Pellny *et al*., 2004; Schluepmann *et al*., 2003).

The feeding of *Arabidopsis* with trehalose elicits strong responses that are not generated by osmoticum control such as sorbitol. Trehalose supplied to the growth medium of seedlings inhibits growth and changes allocation of carbon between roots and shoots (Schluepmann *et al*. 2004; Fritzius *et al*., 2001; Wingler *et al*., 2000). The effects of exogenous trehalose are numerous, yet growth arrest after 100 mM trehalose feeding is due to T6P accumulation. T6P accumulation is not due to phosphorylation of trehalose. Rather, it is likely due to ineffective de-phosphorylation of the endogenously synthesized T6P in the presence of high concentrations of the reaction product *i.e.,* trehalose (Schluepmann *et al*., 2004). In *Arabidopsis*, T6P is essential for modulating efficient carbohydrate utilization during growth (Schluepmann *et al*., 2003). Exogenously applied trehalose strongly induces accumulation of starch in the seedlings' source tissue (shoots), and to a depletion of starch in the sink tissue (roots). The accumulation of starch in the shoots of trehalose-treated seedlings is accompanied by an increased activity of ADP-glucose pyrophosphorylase and an induction of the expression of the ADP-glucose pyrophosphorylase gene, *ApL3* (Wingler *et al*., 2000). These observations suggest that trehalose interferes with carbon allocation to the sink tissues by inducing starch synthesis in the source tissues. Although T6P appears to be synthesized in the cytosol, it targets chloroplast's photosynthetic machinery (Kolbe *et al*., 2005). Taken together, T6P not only has control over carbon allocation but it also controls some steps in chloroplast metabolism.

Sugars have inhibitory effects on photosynthesis or on the expression of photosynthetic genes. In general, low sugar status enhances photosynthesis, reverses mobilization and export, whereas the abundant presence of sugars promotes growth and carbohydrate storage (Koch, 1996). Sugars are important signals molecules that regulate source-sink relation. (Franck *et al*., 2006; Ehness *et al*., 1997; Imail *et al*., 1997; Koch, 1996). A decreased leaf hexose pool may serve as a signal for increased sink demand, and also reduce negative feedback regulation of photosynthesis an effect which has been observed previously in sugarcane (McCormick *et al*., 2008). Documents show that there are several independent sugar signal transduction pathways (Rolland *et al*., 2006). But the exact mechanism of sugar signaling between source-sink relations has yet not been discovered. In this study, the relationship between T6P and expression of two photosynthetic genes has been demonstrated. This is in good accordance with the previous data, where increased levels of hexose correlated with decreased expression of several photosynthesis-related genes (McCormick *et al*., 2008; Franck *et al*., 2006; Krapp *et al*., 1992). Krapp *et al*. (1992) have shown that the addition of glucose to autotrophic cell suspension cultures of *Chenopodium* leads to a large and reversible decrease in the steady state transcript levels of *rbcS* and *ca*b.

Sugars have stimulatory effects on anthocyanin biosynthesis in different organs of several plant species (Weiss, 2000). Anthocyanins are produced in cotyledons or leaves when growing on a sugar containing medium (Mita *et al*., 1997). The previous data have shown that mono- and disaccharides like glucose, fructose, galactose, maltose and sucrose have stimulatory effects on anthocyanin accumulation in *Arabidopsis* seedlings (Teng *et al*., 2005). In this study, trehalose also induced anthocyanin accumulation in *Arabidopsis* seedlings. This effect of trehalose might be mediated through increased levels of sugars like glucose. Frison *et al*., 2007 have recently reported that the *Arabidopsis* trehalase is a plasma membrane-bound enzyme with extra cellular activity but they were unable to assay activity in the membranes. It could therefore be that the enzyme was released from the membrane upon activation. However, further experiments are necessary to obtain information on this aspect.

The current results form chlorophyll measurements are consistent with the previous reports, which suggest that the supply of carbohydrates leads to a reduction in chlorophyll levels in *Arabidopsis* seedlings (Schafer *et al*., 1992).

The previous data obtained from gene-expression profiling did not show expression patterns of the photosynthetic genes after trehalose feeding (Schluepmann *et al*., 2004). However, in this study, expression analysis of these two genes involved in the photosynthetic mechanism displayed an altered gene

expression pattern after 14 days of trehalose feeding. A possible explanation for this discrepancy might be the different assay conditions used by Aghdasi *et al*., 2008; photosynthetic gene repression is not very pronounced after 24 h of trehalose feeding as compared with that after 14 days. The current data of this study show that the exogenous trehalose may reduce expression or stability of *LHCB1* or *PSI-H* mRNA.

Acknowledgments

We thank Dr. Johannes Hanson for his excellent assistance in the Q-PCR experiment, Anja Van Dijken for kindly providing the pBin19 vector and Frits Kindt and Ronald Leito for help with photography.

References

Aghdasi M, Smeekens S, Schluepmann H (2008). Microarray analysis of gene expression patteren in Arabidopsis seedlings under trehalose, sucrose and sorbitol treatment. *Inter J Plant Produc*. 4:1753-1763.

Blazaquez MA, Santos E, Flores CL, Martinez-Zapater JM, Salinas J, Gancedo C (1998). Isolation and molecular characterization of the *Arabidopsis TPS1* gene, encoding trehalose-6-phosphate synthase. *Plant J*. 13: 685-689.

Clough SJ, Bent AF (1998). Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of Arabidopsis thaliana. *Plant J*. 16:735-743.

Ehness R, Ecker M, Godt DE, Roitsch T (1997). Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation 1. *Plant Cell*. 9: 1825-1841.

Elbein AD (1974). The metabolism of alpha, alpha-trehalose. *Adv Carbohydr Chem Biochem*. 30: 227-256.

Elbein AD, Pan YT, Pastuzak I, Carroll D (2003). New insights on trehalose: a multifunctional molecule. *Glycobiology* 13: 17R-27R.

Eastmond PJ, Van Dijken AJ, Speiman M, Kerr A, Tissier AF, Dichinson HG, Jones JD, Smeekens SC, Graham IA (2002). Trehalose-6-phosphate synthase 1 which catalyses the first step in trehalose synthesis is essential for *Arabidopsis* embryo maturation. *Plant J*. 29: 223-235.

Franck N, Vaast P, Génard M, Dauzat J (2006). Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown Coffea arabica. *Tree Physiol*. 26: 517-525.

Frison M, Parrow JL, guillaumot D, Daniete M, Fracois J, Chaumont F, Batoko H (2007). The Arabidopsis Thaliana Trehalase is a plasma membrane-bound enzyme with extra cellular activity. *FEBS*. 21: 4010-4016.

Fritzius T, Aechbacher R, Wiemken A, Wingler A (2001). Identification of ApL3 expression by trehalose complements the starch- deficient Arabidopsis mutant adg2-1 lacking ApL1, the large subunit of ADP-glucose pyrophosphorylase. *Plant Physiol*. 126: 883-889.

Imail I De, Blaipli A, Smith SM (1997). Expression of glyoxylate cycle genes in cucumber roots responds to sugar supply and can be activated by shading or defoliation of the shoot 3. *Plant Mol Biol*. 35:633-640.

Jeffrey S, Humphrey GF (1975). New spectrophotometeric equations determining chlorophyll a, b, c1 and c2 in higher plants, algae and phytoplankton. *Plant Physiol*. 167: 191-194. Koch KE (1996). Carbohydrate- modulates gene expression in plants. *Annu Rev Plant Physiol Plant Mol Biol*. 47: 509-540.

Koch KE (1996). Carbohydrate modulated gene expression. *Annu Rev Plant Biol*. 47: 509-540.

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

Krapp A, Hofmann B, Schafer C, Stitt M (1992). Regulation of the expression of *rbcs* and other photosynthetic genes by carbohydrates: a mechanism for the sink regulation of photosynthesis. *Plant J*. 3: 817-828.

Lunde C, Jensen PE, Hardrup A, Knoetzel J, Scheller HV (2000). The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. *Nature* 408: 613-615.

Leyman B, Van Dijck P, Thevelein JM (2001). An unexpected plethora of trehalose biosynthesis genes in Arabidopsis thaliana. *Trends Plant Sci*. 6: 510-513.

McCormick AJ, Watt DA, Cramer MD (2008). Supply and demand: sink regulation of sugar accumulation in sugarance. *J Exp Bot*. 10: 1-8.

Mita S, Murano N, Akaike Nakamura K (1997). Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that is inducible by sugars. *Plant J*. 11: 841-851.

Muller J, Boller T, Wiemken A (1995). Trehalose and trehalase in plants: recent developments. *Plant Sci*. 112: 9-15.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiol*. 15: 473-479.

Noctor G, Foyer CH (2000). Homeostasis of adenylate status during photosynthesis in a fluctuating environment. *J Exp Bot*. 51: 347-356.

Pellny TK, Ghannoum O, Conroy JP, Schluepmann, H, Smeekens S, Andralojc J, Krause, KP, Goddijn O, Paul MJ (2004). Genetic modification of photosynthesis with *E.coli* genes for trehalose synthesis. *Plant Biotechnol J*. 2: 71-82.

Ramon M, Rolland F, Thevelein JM, Van Dijck P, Leyman B (2007). *Abi4* mediate the effects of exogenous trehalose on *Arabidopsis* growth and starch breakdown. *Plant Mol Biol*. 63: 195-206.

Rasmussen R (2000). Quantification on light cycler. In Rapid cycle Real-Time PCR, methods and applications. Springer press, Heidelberg, 21-34.

Rolland F, Baena-Gonzales E, Sheen J (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu Rev Plant Biol*. 57: 657-709.

Ruban AV, Young AJ, Pascal AA, Horton P (1994). The effects of illumination on the xanthophyll composition of the photosys-

tem II light-harvesting complexes of Spinach thylakoid membranes. *Plant Physiol*. 104: 227-234.

Schafer C, Simper H, Hofmann B (1992). Glucose feeding results in coordinated changes of chlorophyll content, ribulose-1,5-bisphosphate carboxylase-oxygenase activity and photosynthetic potential in photoautrophic suspension cultured cells of Chenopodium rubrum. *Plant Cell Environ*. 15: 343-350.

Schluepmann H, Pellny T, Van Dijken A, Smeekens S, Paul MJ (2003). Trehalose-6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis Thaliana*. *Proc Natl Acad Sci USA*. 100: 6849-6854.

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.

Shima S, Matsui H, Tahara S, Imai R (2007). Biochemical characterization of rice trehalose-6-phosphate phosphatases supports distinctive functions of these plant enzymes. *FEBS J*. 274: 1192.

Smeekens S (2000). Sugar-induced signal transduction of anthocyanin in plants. *Annu Rev Plant Physiol Plant Mol Biol*. 51:49-81.

Teng S, keurentjes J, Bentsink L, Koornneef M, Smeekens S (2005). Sucrose-specific induction of anthocyanin biosynthesis in *Arabidopsis* requiers the MYB75/PAP1 gene. *Plant Physiol*.139: 1840-1852.

Vinit G, Fourrier N, Bowyer JR, Lopez-Juez E (2005). *Arabidopsis* mutants with defective plastids are impaired primarily in the photocontrol of expression of photosynthesis-associated nuclear genes. *Plant Mol Biol*. 57: 343-357.

Vogel G, Aeschbacher RA, Muller J, Boller T, Wiemken A (1998). Trehalose-6-phosphate phosphatases from *Arabidopsis thaliana*: identification by functional complementation of the yeast tps2 mutant. *Plant J*. 13: 673-683.

Wiess D (2000). Regulation of flower pigmentation and growth: numerous signaling pathways control anthocyanin synthesis in expandind petal. *Plant Physiol*. 110: 152-157.

Wingler A, Fritzius T, Wiemken A, Boller T, Aeschbacher RA (2000). Trehalose induces the ADP-glucose pyrophosphorylase gene, ApL3, and starch synthesis in *Arabidopsis*. *Plant Physiol*. 124: 105-114.

Zentella R, Mascorro-Gallardo JO, Van Dijck P, Folch-Mallol J, Bonini B, Van Vaeck C, Gaxiola R, Covarrubias AA, Nieto-Sotelo J, Thevelein JM, Iturriaga GA (1999). Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast tps1 mutant. *Plant Physiol*. 119: 1473-1482.