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RESEARCH PAPER

Functional characterization of the *thi1* promoter region from *Arabidopsis thaliana*

Denise Teixeira Ribeiro[1], Leonardo Paiva Farias[1], Juliana Dantas de Almeida[2], Priscila Mayumi Kashiwabara[1], Alberto F. C. Ribeiro[3], Marcio C. Silva-Filho[2], Carlos Frederico Martins Menck[4] and Marie-Anne Van Sluys[3,*]

[1] *Depto. de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-900 São Paulo, SP, Brazil*

[2] *Depto. de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Av. Pádua Dias, 11, 13400-970 Piracicaba, SP, Brazil*

[3] *Depto. de Biologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-900 São Paulo, SP, Brazil*

[4] *Depto. de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374, 05508-900 São Paulo, SP, Brazil*

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Abstract

The *Arabidopsis thaliana* THI1 protein is involved in thiamine biosynthesis and is targeted to both chloroplasts and mitochondria by N-terminal control regions. To investigate *thi1* expression, a series of *thi1* promoter deletions were fused to the β-glucuronidase (GUS) reporter gene. Transgenic plants were generated and expression patterns obtained under different environmental conditions. The results show that expression derived from the *thi1* promoter is detected early on during development and continues throughout the plant's life cycle. High levels of GUS expression are observed in both shoots and roots during vegetative growth although, in roots, expression is restricted to the vascular system. Deletion analysis of the *thi1* promoter region identified a region that is responsive to light. The smallest fragment (designated Pthi322) encompasses 306 bp and possesses all the essential signals for tissue specificity, as well as responsiveness to stress conditions such as sugar deprivation, high salinity, and hypoxia.

Key words: GUS expression, promoter analysis, protein targeting, sugar modulation, *thi1*, thiamine biosynthesis, tissue expression pattern.

Introduction

Thiamine pyrophosphate (TPP), the active form of vitamin B-1, is a key cofactor of the essential enzymes involved in carbon metabolism. TPP is involved in the transfer of aldehyde groups during decarboxylation steps (Hohmann and Meacock, 1998). Plants are thiamine prototrophic, however, the biosynthetic pathway of thiamine is not yet well understood. Bacteria and fungi models suggest that thiamine-P is formed by the condensation of two independently synthesized components: 5-hydroxymethyl-2-methyl-4-amino-pyrimidine pyrophosphate (HMP-PP) and 5-hydroxyethyl-4-methylthiazole phosphate (HET-P) (Spenser and White, 1997). In plants, chloroplasts were demonstrated to be the site for the synthesis of the thiazole moiety (Julliard and Douce, 1991), while in yeast, evidence points to mitochondria (Belanger *et al*., 1995; Machado *et al*., 1996).

The THI1 protein, named thiazole biosynthetic enzyme, belongs to a conserved protein family that encompasses orthologues from other species (Choi *et al.*, 1990; Manetti *et al.*, 1994; Praekelt *et al.*, 1994; Belanger *et al.*, 1995; Jacob-Wilk *et al.*, 1997; Ribeiro *et al.*, 1996). The gene was originally isolated from an *A. thaliana* cDNA library after complementation with *Escherichia coli* mutants deficient in DNA repair and stress-tolerance mechanisms (Machado *et al.*, 1996). As well as increasing the survival rates of

* To whom correspondence should be addressed. Fax: +55 11 3091 7547. E-mail: mavsluys@ib.usp.br

bacteria defective in DNA repair mechanisms, the *thi1* cDNA was found to restore the mitochondrial stability of the yeast *Thi4* mutant after treatment with DNA-damaging agents (Machado *et al.*, 1996, 1997).

THI1 protein is targeted simultaneously to mitochondria and chloroplasts by a post-transcriptional mechanism (Chabregas *et al*., 2001, 2003). This dual targeting mechanism is rare in proteins directed to more than one compartment (for a review see Silva-Filho, 2003). Molecular characterization confirmed that *thi1 is* a single copy nuclear gene, with a transcription initiation site located 39 bp upstream to the first ATG and encodes a single 1.3 kb transcript (Chabregas *et al*., 2001). The predicted protein is 349 amino acids in length, containing a typical chloroplast transit peptide and a mitochondrial presequence-like structure at the N-terminus, enabling dual organellar targeting.

Recently, the thiamine auxotrophic *Arabidopsis* tz-201 mutant line (Feenstra, 1964) was shown to possess a point mutation in a conserved region of the *thi1* gene, which hinders complementation of the *thi4* yeast strain (Papini-Terzi *et al*., 2003). In the same work, it was demonstrated that the expression of *thi1* mRNA is reduced in the dark, is more pronounced in shoots than in roots, but is not affected by thiamine-deprivation in both wild-type plants or the mutant line, as reported for the fungal orthologue.

Here, a functional analysis of the *thi1* promoter region was performed to determine the expression pattern of the gene and its responsiveness to various stress conditions. A series of 5′ *thi1* promoter deletions was constructed, fused to the GUS reporter gene and introduced into *Arabidopsis* via *Agrobacterium tumefaciens* stable transformation. GUS activity was assayed using histochemical and fluorimetric analyses and showed that the *thi1* promoter has a broad expression pattern being detected in all organs and at different stages throughout the plant's life cycle. Nevertheless, it was shown to be more active in shoot tissues than in roots of mature plants. Expression is affected by high salinity, sugar deprivation and flooding. The expression pattern was the same for all promoter deletion constructs. Therefore, the smallest construct (306 bp) harbours the signals required for gene expression. A *cis*-acting light response element is located between −1277 to −608 in the promoter region. The results presented in this work provide the first molecular basis of transcriptional regulation of an essential thiamine biosynthetic gene.

Materials and methods

DNA constructs

The *thi1* promoter fragments utilized in this study comprise of regions from position −16 up to positions −1277 (Pthi1277), −608 (Pthi608), and −322 (Pthi322) relative to the translation initiation site. These were obtained by PCR amplification of the *A. thaliana* wild-type (Landsberg *erecta* ecotype) genomic clone (Papini-Terzi *et al*., 2003). After subcloning and sequencing, promoter-containing inserts were transferred to pCAMBIA 1281Z (http://www.cambia.au) containing the β-glucuronidase reporter gene (*GUS*) and the hygromycin selection gene. As a positive control, the CaMV 35S promoter fragment from positions −645 to +35 relative to the transcription start site, was subcloned into the same vector for comparative purposes. A negative control, named Pthi472, was prepared and contained a fragment encompassing positions −322 to +150 of the original *thi1* genomic clone in the antisense orientation.

To analyse the subcellular location of the THI1 protein in a homologous and stable system in *Arabidopsis*, a chimaeric gene was prepared using the green fluorescent protein (GFP) encoding gene. THI1-GFP fusion protein was obtained by PCR amplification of the entire *thi1* coding sequence from the p(SK+)*thi1* vector (Machado *et al*., 1996) and its stop codon removed with the following primers: 5′-CCC*GGATCC*ATGGCTGCCATAGC and 5′-GGG*TCTAGA*AGCATCTACGGTTTCAGC, carrying the *Bam*H1 and *Xba*I sites, respectively (indicated in italics/underlined). The amplified DNA fragment was cloned into the corresponding sites of the pGEM–Pthi1277 vector, which carries the *thi1* promoter region, to yield the vector p1277-thi1. Subsequently, the GFP gene along with the *nos* terminator region was amplified by PCR from the vector pBC-GFP (Davis and Vierstra, 1998), with the following primers: 5′-CCCTCTAGAATGAGTAAAGGAG and 5′-GATCATGCGA*GCGGCCGC*CTGCAGGTCAAT, carrying the *Xba*I and *Not*I sites, respectively (indicated in italics). This fragment was cloned into the p1277-thi1 vector, previously digested with *Xba*I and *Not*I, yielding the p1277-*thi1*GFPnos vector and was subsequently checked by sequencing. Finally, the gene construct encompassing the *THI1* regulatory sequence fused to the chimaeric gene composed of *THI1* linked to *GFPnos* was isolated by a double restriction with *Kpn*I and *Pst*I and subcloned into the plant expression vector pCAMBIA 2300 (Roberts *et al*., 1998), yielding the vector pCAMBIA-1277- thi1GFPnos.

All *Arabidopsis* lines obtained in this study were generated in the Wassilewskija (Ws) ecotype. Plants were grown in appropriate controlled culture rooms between 22 °C and 25 °C, 50% average relative humidity, and with a 12/12 h photoperiod. For *in vitro* culture, seeds were surface-sterilized in 5% sodium hypochloride solution for 8 min, washed five times in sterile water, and spread onto MS medium (Sigma modified basal salt mixture; M0153, Nitsch vitamins; pH 6.5), solidified with 6.5 g l^{-1} phytagar (Invitrogen, Eggenstein, Germany), containing 20 g l^{-1} sucrose (MS20). The plants were grown for 14 d and subsequently transferred to larger containers for a further 2 weeks. Plants were then transplanted to soil mixed with vermiculite (1:1 v:v) and a mineral solution (5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 1 mM Fe EDTA, 46 μM H_3BO_3, 9.14 μM $MnCl_2.4H_2O$, 8.06 μM $ZnCl_2$, 0.37 μM $CuCl_2$, 0.1 μM $NaMoO4.2H_2O$) was supplied weekly.

Arabidopsis *transformation and selection*

Wild-type seeds were spread directly onto sterile soil mixed with vermiculite (1:1 v:v) and transformation was performed by the infiltration method (Clough and Bent, 1998) using the *A. tumefaciens* strain LB4044 carrying the constructs described above. Seeds were collected and germinated *in vitro* on selective media (MS20 containing 20 μg ml^{-1} hygromycin). Resistant plants (T_0) were cultivated (more than 60 per construct). Seeds were harvested and a second screen for hygromycin resistance was carried out on the T_1 generation. The lines with Mendelian 3:1 hygromycin resistance segregation were selected for further experimentation. At least five lines carrying the CaMV 35S construct and 10 lines carrying each *thi1* construct (Pthi1277, Pthi608, and Pthi322) were obtained. The results presented in this study are derived from T_2 plant lines.

Northern blot

Total RNA was extracted from rosette leaves of 21-d-old wild-type and transgenic plant lines grown *in vitro* using TRIzol reagent

(Invitrogen, Eggenstein, Germany), as recommended by the manufacturer. Around 5 μg of RNA was denatured and loaded onto a 6.7% formaldehyde/1.5% agarose denaturing gel. After electrophoresis, the RNA samples were transferred to nylon membranes (GeneScreen Plus, NENTM Life Science Products) and probed with a PCR amplified GUS 240 bp fragment (primers: 5′-CCTTACGCTGAA-GAGATGCT-3′ and 5′-GGCAATACTCCACATCACCA-3′) or *THI1* cDNA, under high stringency conditions (7% SDS, 1 mM EDTA, 0.5 M sodium phosphate buffer pH 7.4 at 65 °C) for 18 h. The amount of RNA transferred to the membrane was normalized utilizing the 16S rDNA as probe. Radioactive probes were prepared using labelled ^{32}P αdATP (25 μCi), DNA polymerase I and random primers (Sambrook *et al*., 1989).

Experimental conditions

Plants were cultivated *in vitro* as described above, and harvested after 7, 14, 21, and 29 d for assaying GUS activity. The selective antibiotic was eliminated on the 14th day, when resistant plants were transferred to larger containers. For light response experimentation, seeds were imbibed in Petri dishes with selective medium (MS20 with 20 μg ml^{-1} hygromycin), maintained under light. On the third day the germinated seedlings were transferred to new media and kept under light or wrapped in aluminium foil for 11 d. A subset of plants grown in the dark was transferred back to light 3 d prior to harvesting.

Stress conditions were applied to hygromycin selected 11-d-old plantlets, transferring them either to MS20 liquid media, where only the roots were submerged (flooding conditions), to MS20 fresh solid media with 100 mM NaCl (salt/osmotic stress), to MS media without sucrose (sugar deprivation), or to MS20 fresh media with standard composition (control plants). Plants were kept for 3 d under the above conditions prior to measuring GUS activity.

GUS assays

GUS enzyme activity in transgenic *Arabidopsis* seedlings was determined fluorimetrically according to Jefferson (1987). The protein concentration was determined utilizing BSA as a standard protein as described by Bradford (1976). Histochemical staining of GUS activity was performed as described by McCabe *et al*. (1988). Photomicrographs were taken with a Nikon Optiphot microscope.

Confocal microscopic analysis

Confocal microscopy was performed using a Zeiss LSM 410 laser scanning confocal imaging system. For GFP detection, excitation was at 488 nm and detection between 505 and 555 nm.

Search for transcription factor binding sites on the THI1 promoter sequence

The 1277 bp 5′-sequence upstream the initial ATG was scanned for probable transcription factor binding sites with the aid of the 'MatInspector V2.2' program (http://transfac.gbf.de/cgi-bin/matSearch; Quandt *et al*., 1995).

Results

*P*thi1 *expression pattern*

Temporal and tissue specificity of *thi1* expression was determined by performing GUS histochemical assays on transgenic plant lines harbouring the different *thi1* promoter–GUS fusion constructs (Fig. 1). Expression is observed in most tissues from the early stages after germination to the end of the plant's life cycle. All constructs presented the same pattern, and the results are illustrated with images from plants containing the entire *thi1* promoter, Pthi1277 (Fig. 1C–O). Positive (P35S) and negative (Pthi472) controls are presented in Fig. 1B and Fig. 1A, respectively. GUS activity in young plants was detected in roots and shoots including cotyledons (Fig. 1E–G), leaves (Fig. 1H–J), and hypocotyls. Strong staining is also observed in vascular tissues and in the apical meristematic region (Fig. 1N). The radicle is completely stained in emerged seedlings (Fig. 1D). However, roots from 14-d-old plants were stained preferentially in the vascular tissues (Fig. 1O), whilst the ground tissue and root cap remained unstained. Expression was also observed in the inflorescence (Fig. 1K), siliques (Fig. 1L–M), and in embryos (Fig. 1C).

Root expression of the *thi1* gene is limited to the vascular tissue, consistent with a complementary study where the full *thi1* promoter drove the expression of a chimaeric gene composed of the *thi1* full-length cDNA fused to GFP encoding gene (Fig. 2). The results clearly showed that the THI1-GFP fusion protein is restricted to vascular tissue and targeted to plastids.

Further analyses on shoot and root tissues of transgenic lines were carried out using fluorimetric assays in order to determine the promoter strength and expression pattern relative to the constitutively expressed 35S promoter. Results presented in Fig. 3A confirm previous data (Papini-Terzi *et al*., 2003) where shoot tissues show higher expression levels. GUS expression driven by the *thi1* promoter is 3–4-fold higher in shoots than roots, while the opposite is observed when GUS is under the control of the 35S promoter. The fact that the smallest construct (Pthi322) determines the same expression pattern as that of Pthi1277 suggests that it contains the tissue-specific response elements. Thus, the *thi1* promoter is strongly active in shoots, and is comparable to the constitutive CaMV 35S promoter, but is less active in roots. As a control to the fluorimetric assay, expression profiles using northern blot hybridization of 16S rDNA, the *thi1* endogenous gene, and the GUS gene were determined for the plant lines utilized in the fluorimetric assays (Fig. 3B). Transgenesis did not affect endogenous gene expression when comparing *thi1* and 16S rDNA gene expression between transgenic and wild-type plant lines.

Light modulation of GUS expression by P35S and Pthi1

Based on previous results, experiments were designed to evaluate light modulation over GUS gene expression driven from the 35S promoter and the three *thi1* promoter deletion fragments. Fourteen-day-old plants were either kept in the dark, a 12/12 h light regime, or subjected to 8 d dark before being transferred to light for the remaining 3 d prior to GUS fluorimetric assays (Fig. 4). *thi1* promoter activity in light-grown plants is 2-fold higher than in dark-grown plants. Dark-to-light transfer promotes a light adaptation, but did not reach the levels achieved by light-grown plants. A distinct behaviour was observed for 35S::GUS expression

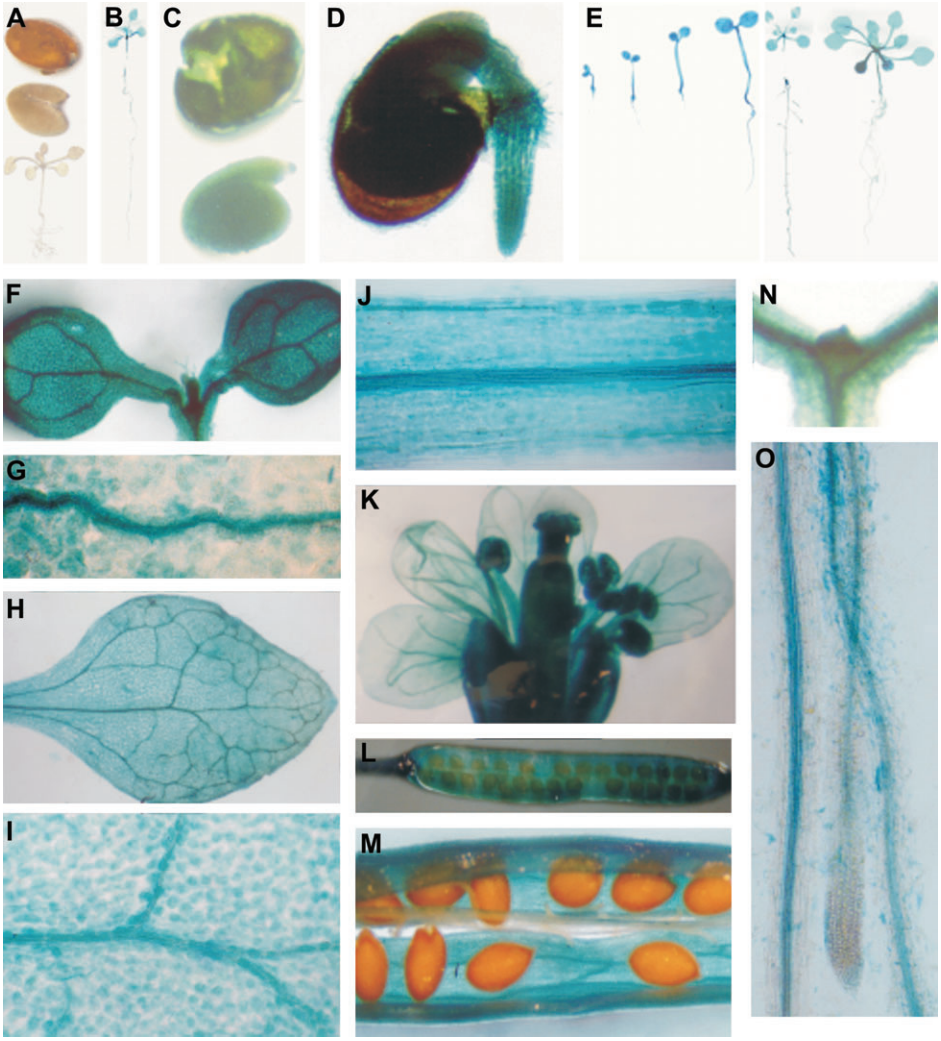

Fig. 1. *In situ* localization of thi1–GUS activity in *Arabidopsis* transgenic plants. (A) Negative control (Pthi472+GUS)–seed coat, embryo, and 14-d-old plant; (B) positive control (P35S+GUS)–14-d-old plant; (C) Pthi1277 transgenic plant (and the following images)–seed coat and embryo; (D) germinating seedling; (E) vegetative development: 2, 3, 5, 7, 14, and 21-d-old plants; (F) 7-d-old plant with stained cotyledons, leaf primordia, and hypocotyls; (G) cotyledon detail; (H) 14-d-old plant leaf; (I) leaf detail; (J) petiole; (K) flower; (L) silique; (M) open silique detail; (N) apical meristematic region; (O) 14-d-old plant roots.

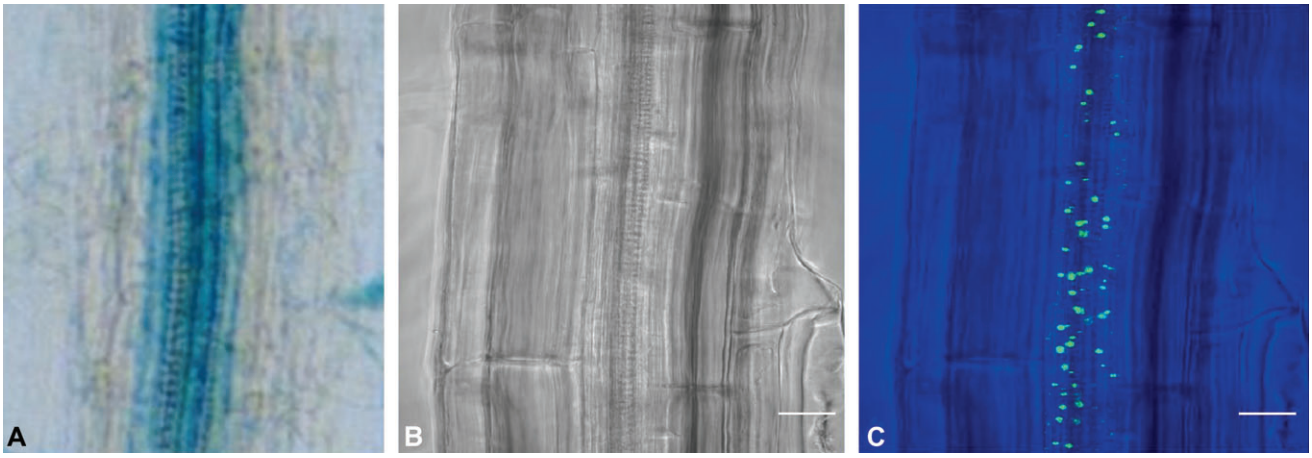

Fig. 2. Root tissue expression pattern of *thi1* promoter. (A) Histochemical expression of the GUS reporter gene driven by the Pthi1277 promoter region. (B, C) confocal analyses of the GFP reporter gene driven by Pthi1277 fused to the *thi1* cDNA and GFP. GFP is found in the plastids.

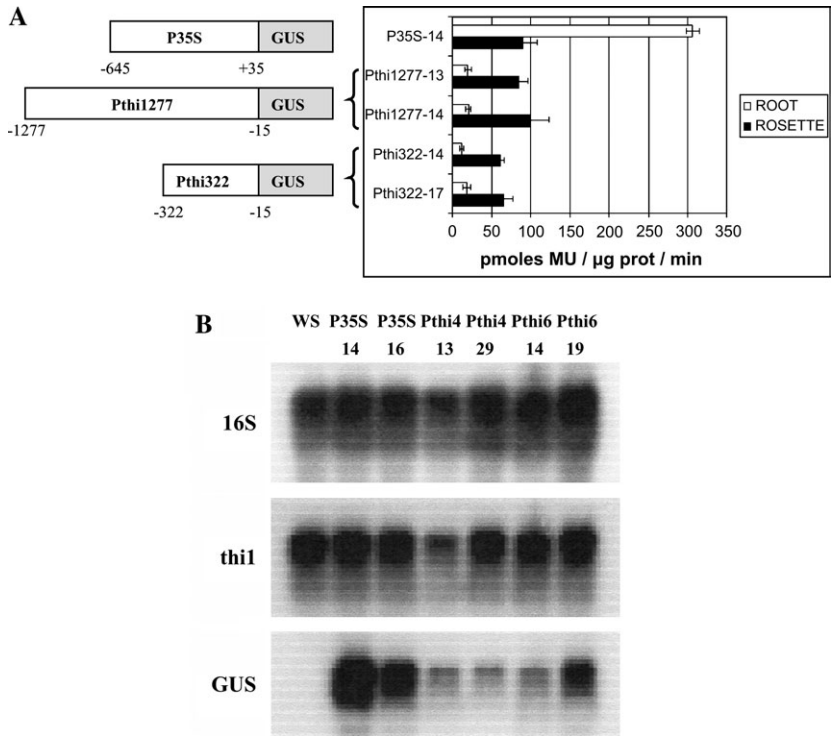

Fig. 3. Quantitative expression of GUS activity in shoots and roots from five independent transgenic *Arabidopsis* lines. (A) Fluorimetric assays were performed with a pool of 20 14-d-old plants from the P35S-14 line, the Pthi1277-13 and Pthi1277-14 lines, and the Pthi322-14 and Pthi322–117 lines. Error bars represent the standard deviation obtained from three independent experiments. (B) Northern blot hybridization of total RNA with the indicated radioactive labelled probes on the left.

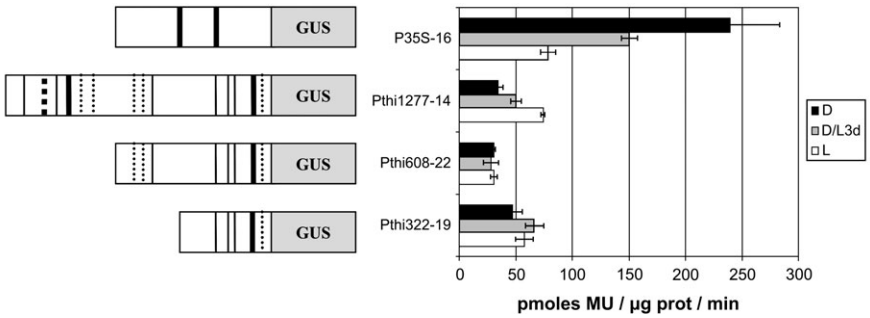

Fig. 4. Light modulation of the *thi1* promoter. Seeds from plant lines containing different portions of the *thi1* promoter region were germinated in light with a 12/12 h photoperiod for 3 d. Plants were *in vitro*-cultured for 11 d under the same light conditions (L), dark (D) or cultured in the dark and transferred to light for the last 3 d prior to harvesting (D/L3d). Protein extracts from a pool of 20 plants were assayed for GUS activity as indicated in the Materials and methods. Error bars represent the standard deviation obtained from three independent experiments. Schematic representation of the constructs is shown on the left. Transcription factor motifs related to light modulation are represented by: thin line, SBF-1; thick line, P-flavoprotein; square line, MYBPH3; star line, GAMYB.

with higher activity in the dark-grown plants than in light (2–3-fold). Lack of the first 608 bp in the 5′ portion of the *thi1* promoter portion appears to abolish light regulation as Pthi608 and Pthi322 presented the same expression pattern in the three conditions.

Pthi1 is responsive to sugar deprivation and to increased salinity

In order to evaluate the effect of stress conditions on the expression pattern of the *thi1* promoter, plants were subjected to three distinct environmental conditions: sugar deprivation, salinity, or flooding. After 3 d treatment, plants were assayed for GUS activity in shoots (Fig. 5A), and roots (Fig. 5B), respectively. Interestingly, root tissues were more responsive to all treatments than were shoots. Flooding conditions increased GUS expression 2.5-fold in roots, but not in shoots, irrespective of the Pthi1 construct present in the transgenic plant lines. Salt stress increased GUS expression 2-fold in shoots and 3.5-fold in roots. Sugar deprivation had the most significant change in the expression pattern

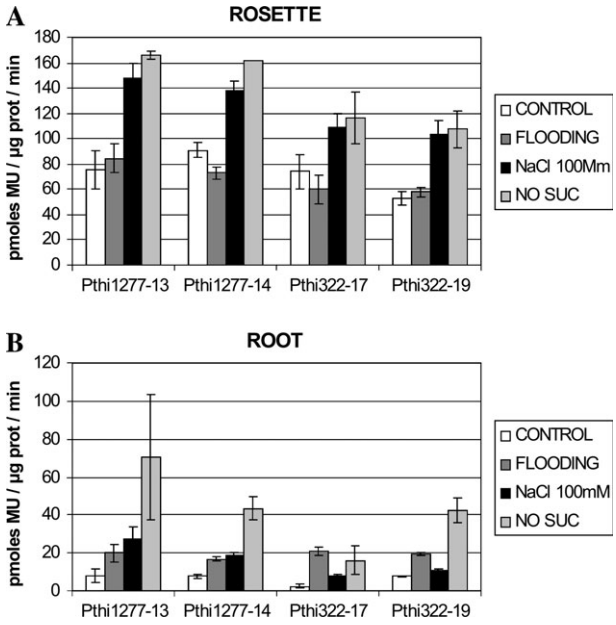

Fig. 5. Effect of stress conditions on GUS activity driven from different *thi1* promoter constructs. Assays were performed on rosettes and roots from five transgenic *Arabidopsis* lines. Plants were cultured *in vitro* for 11 d, and then transferred to liquid media with the roots submerged (FLOODING), or new solid media containing 100 mM NaCl or without sucrose (NO SUC). Protein extracts from rosettes (A) or roots (B) from a pool of 20 plants were assayed for GUS activity as indicated in the Materials and methods. Error bars represent the standard deviation between three independent experiments.

where GUS expression was up-regulated 6-fold in roots and only 2-fold in shoots.

Discussion

THI1 is involved in the biosynthesis of the thiamine cofactor, necessary for the functioning of important carbon metabolic enzymes. A functional characterization of the *thi1* gene promoter region is presented here, based on transgenic plants carrying the GUS reporter gene fused to different *thi1* promoter fragments. Activity was assayed *in vivo* using histochemical and fluorimetric methods. Quantitative measurements by means of fluorimetric assays are more sensitive than histochemical assays to determine whether a particular environmental condition affects the level of gene expression (Jefferson, 1987). Comparative analysis between transgenic plant lines harbouring the 35S promoter and Pthi1277 reveals that the latter is a strong promoter. Considering that THI1 is involved in vitamin biosynthesis, it is interesting to note that it is under the control of a constitutive and highly active promoter. Thiamine is required in trace amounts, but it has been shown that enzyme–coenzyme complexes, such as pyruvate dehydrogenase from plant mitochondria are unstable during purification, compared with what has been observed for the mammalian pyruvate dehydrogenase complex (Douce and Neuburger, 1989). If they are also unstable *in vivo*, the synthesis of thiamine should be continuous and dependent on a significant rate of synthesis. Indirect evidence showed a high level of exogenous thiamine requirement to restore normal metabolism in mutants of *Pisum sativum* with altered pyrimidine moiety synthesis (Proebsting *et al*., 1990).

Although *thi1* promoter activity is highest in shoots, roots do show some GUS activity, consistent with *thi1* mRNA expression data (Papini-Terzi *et al*., 2003). As discussed, this difference is not due to light regulation but rather to promoter tissue specificity, as the plants used were cultivated *in vitro* and roots were also exposed to light. These results suggest that tissue-specific control is located in the 3′ portion of the promoter region, specifically the downstream position −322 relative to the translation initiation site. The 35S promoter also confers a tissue-specific expression, where the activity in roots is higher than in shoots. This tissue specificity is probably associated with the presence of two AS-1 motifs in the 35S promoter, whereas only one is found in Pthi1277 (position −112). At the subcellular level, THI1 protein is compartmentalized to plastids (Chabregas *et al*., 2003), where it participates in the synthesis of the thiazole moiety in chloroplasts (Julliard and Douce, 1991). Therefore, it is expected that *thi1* would show higher expression levels in shoots, reinforced by the fact that roots are not thiamine auto-sufficient. Nevertheless, the *thi1* promoter is active in most plant tissues, similar to other constitutive promoters.

On the other hand, GFP fusion analyses suggest that Pthi1 is active in roots but expression is restricted to the vascular system, more precisely, the protein is targeted solely to the plastids. This result confirms that although roots are dependent on a thiamine supply from the photosynthetic tissues, they express one of the key thiamine biosynthetic proteins, supporting the hypothesis of a dual role for THI1 as proposed previously and suggests a partitioning of thiamine synthesis in plants. In contrast to previous results (Chabregas *et al*., 2003), analysis of several transformants harbouring the THI1–GFP fusion protein did not show a mitochondrial GFP expression pattern. It is interesting to note that a similar GFP fusion protein made by the targeting sequence of the thiazole biosynthetic enzyme homologue from *Citrus sinensis* was associated with small vesicles that adhered to the outer chloroplast membrane, but not to mitochondria (Escobar *et al*., 2003). Together, this suggests that the size and nature of the mature GFP fusion might interfere with protein translocation to mitochondria. Alternatively, the imported protein may not be at a detectable level. Inefficient dual targeting of GFP to mitochondria and chloroplasts mediated by other proteins have also been reported (Beardslee *et al*., 2002; Goggin *et al*., 2003).

The *thi1* promoter is under daylight control, along with its mRNA accumulation as previously described

(Papini-Terzi *et al*., 2003). Plants submitted to dark conditions for 11 d after germination presented reduced levels (2-fold) of GUS activity. When dark-grown plants were transferred to light conditions for 3 d, GUS activity increased. This suggests that light modulates activity of the *thi1* gene whereby the gene is down-regulated in the dark and up-regulated in the presence of light. Furthermore, this regulatory mechanism is lost when the 5′ portion of the promoter region is removed, as demonstrated with the Pthi608 construct. There are some transcription factor binding motifs in this region related to light control, such as MYB, SBF-1, and GT-1 (Harrison *et al*., 1991; Solano *et al*., 1995), and regulation is probably dependent on the interaction of all these factors.

Homologous genes encoding thiazole biosynthetic enzyme described in fungi (*THI4*, *sti35*, and *nmt1*) are subjected to stringent control. Absence of thiamine in the medium is a strong activator of gene expression. The *Fusarium* orthologue *sti35* (Choi *et al.*, 1990) was originally cloned as a stress-induced gene. Expression of the plant orthologues is not affected by thiamine availability but is associated with cells undergoing particular developmental pathways such as nodule differentiation (Ribeiro *et al*., 1996) and ethylene-induced fruit maturation (Jacob-Wilk *et al*., 1997). In order to study environmental factors other than light that could affect THI1 expression, plant lines were subjected to flooding conditions, salt tolerance, and sugar deprivation. Flooding stress was mimicked by submerging the plant roots in liquid medium for 3 d, which generates an hypoxic condition in this organ. An increase in GUS activity was observed in roots after the treatment, suggesting that *thi1* expression is necessary during flooding. Roots normally obtain sufficient oxygen for aerobic respiration, however, when the supply of O_2 is insufficient, roots begin to ferment pyruvate, a much less effective energy production pathway than respiration. When maize roots are submitted to flooding, most protein synthesis ceases except for some enzymes identified as components of the glycolytic and fermentation pathways (Sachs *et al*., 1980). Higher levels of thiamine may also be required in this metabolic deviation, as it acts as a cofactor with pyruvate decarboxylase involved in the fermentative route. However, the possibility of a role for THI1 in resisting oxidative stress cannot be discarded, and constitutes a clue to a DNA damage/tolerance function (Machado *et al*., 1997).

The *thi1* promoter was also responsive to salt stress, doubling GUS activity after 3 d in high salinity conditions in both shoots and in roots. This was not observed for the 35S promoter. An ABRE (abscisic acid responsive element) motif was found in the 3′ portion of the *thi1* promoter (−110 position). This motif is commonly found in genes involved in plant responses and processes mediated by ABA, like drought or high salinity responses. As the Pthi322 construction is also responsive, it is likely that this motif is functional.

The *thi1* promoter was activated by sugar deprivation and this response was dependent on the 322 bp 3′ portion. However, these motifs in the *thi1* promoter are located upstream of position −322 and are hence probably not involved in this response. A motif, AATAGAAAA, conserved among sucrose-regulated genes, was found at position −484 in the *thi1* promoter, with two altered bases (AATAGA**GC**A). Sugar deprivation redirects the plant metabolism to photosynthesize efficiently in order to support the need for hexoses and *thi1 is* probably coordinately regulated with other genes during changes in thiamine requirement.

Much effort has been dispensed in order to elucidate the functioning of this intriguing gene in thiamine biosynthesis and DNA damage/tolerance. This study's approach presents new data on *thi1* regulation and attempts to illuminate the biochemical network with which *thi1* participates in. It is demonstrated that the *thi1* promoter is strongly and ubiquitously expressed. It behaves like other constitutive promoters from essential genes that participate in central metabolism, with its gene product being required throughout the plant life cycle. Nevertheless, *thi1* promoter activity was shown to be up-regulated under various environmental conditions where metabolic alterations are triggered, probably being co-regulated with genes encoding enzymes that require thiamine as a cofactor.

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