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Transcriptional regulation of the ADP-glucose pyrophosphorylase isoforms in the leaf and the stem under long and short photoperiod in lentil

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ABSTRACT

ADP-glucose pyrophosphorylase (AGPase) is a key enzyme in plant starch biosynthesis. It contains large (LS) and small (SS) subunits encoded by two different genes. In this study, we explored the transcriptional regulation of both the LS and SS subunits of AGPase in stem and leaf under different photoperiods length in lentil. To this end, we first isolated and characterized different isoforms of the LS and SS of lentil AGPase and then we performed quantitative real time PCR (qPCR) to see the effect of photoperiod length on the transcription of the AGPase isforms under the different photoperiod regimes in lentil. Analysis of the qPCR results revealed that the transcription of different isoforms of the LSs and the SSs of lentil AGPase are differentially regulated when photoperiod shifted from long-day to short-day in stem and leaves. While transcript levels of LS1 and SS2 in leaf significantly decreased, overall transcript levels of SS1 increased in short-day regime. Our results indicated that day length affects the transcription of lentil AGPase isoforms differentially in stems and leaves most likely to supply carbon from the stem to other tissues to regulate carbon metabolism under short-day conditions.

1. Introduction

ADP-glucose pyrophosphorylase (AGPase) is a key regulatory allosteric enzyme involved in starch biosynthesis in higher plants. AGPase catalyzes a rate-limiting reversible reaction and controls carbon flux in the α-glucan pathway by converting glucose-1-phosphate and ATP to ADP-glucose and pyrophosphate using Mg^{2+} as a cofactor [1–4]. The regulation of almost all AGPases in photosynthetic tissues depends on the ratio of 3-phosphoglyceric acid to inorganic phosphate (3-PGA/P_i); while 3-PGA functions as the main stimulator, P_i inhibits the activity of the enzyme [5,6]. Plant AGPases consist of pairs of small (SS, or α) and large (LS, or β) subunits, thereby constituting a heterotetrameric structure ($\alpha_2\beta_2$). These two subunits are encoded by two distinct genes [7–9], and the sequence identity between the subunits is 40–60%, suggesting a common ancestral gene [7,9]. The molecular weights of tetrameric AGPases range from 200 to 240 kDa depending on the tissue and the plant species. Specifically, the molecular weights of the LS and SS in potato tuber AGPase are 51 and 50 kDa, respectively [8]. The SS and the LS have different roles in enzyme functionality [8]; the SS was shown to have both catalytic and regulatory functions, whereas the LS is mainly responsible for regulating the allosteric properties of SS [10–12]. These results were also supported by studies demonstrating that LS was incapable of assembling into a catalytically active oligomeric structure, whereas SS was able to form a homotetramer with catalytic properties [10,13]. However, this SS homotetramer was defective in terms of catalysis and regulation; it required higher concentrations of 3-PGA for activation and was more sensitive to P_i inhibition. These results suggested that the LS is essential for efficient enzyme function [1,10,14,15]. Recent studies have indicated that the LS may bind to glucose 1-phosphate (G1-P) and ATP. The binding of the LS to these substrates may allow the LS to interact cooperatively with the catalytic SS in binding substrates and effectors and, in turn, influence net catalysis [16–18]. A recent study suggested that one of the AGPase LS isoforms from tomato exhibits catalytic activity as a monomer [19]. Additionally, specific regions from both the LS and the SS were found to be important for subunit association and enzyme stability [15,20–23].

The relationship between carbon assimilation and utilization has been studied in detail and greatly affects plant growth and yield. Studies using Arabidopsis subjected to either artificially extended or shortened day length indicated that plants respond globally to different light regimes and are able to adjust their carbon metabolism and growth by regulating enzymes that participate

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in both starch biosynthesis and turnover [24,25]. This regulation occurs on both transcriptional and posttranslational level [26,27]. When Arabidopsis was exposed to an artificially extended night period, plant growth decreased due to starch depletion [28]. Additionally, a similar experiment was performed using a mutant of Arabidopsis that cannot accumulate starch. This mutant exhibited a retarded growth phenotype under a normal light–dark cycle, and its transcriptional profile was altered compared with that of a wild-type plant [28,29]. The aforementioned studies and other previous studies have revealed that AGPase is the key enzyme in starch biosynthesis, and AGPase activity is greatly reduced during an artificially extended night due to posttranslational modifications [28]. Additionally, a recent transcriptome analysis revealed that LS2 of *Hordeum vulgare* AGPase is one of the enzymes that is differentially expressed during a 15-h light/9-h dark regime [30].

In this study, we explored the effect of photoperiod length on AGPase transcription; specifically, we examined the transcription of the LS and SS isoforms in a long-day plant, lentil. We analyzed the transcription profiles of isolated AGPases from lentil growing under long-day (16-h light/8-h dark) and short-day (8-h light/16-h dark) conditions by qPCR. Our results indicated that plants respond to the short photoperiod by decreasing LS1 AGPase transcription by approximately 70% in leaves, while LS2 transcription was increased by approximately 20-fold in leaves under a short-day regime at 10 h. Additionally, overall transcription of lentil SS1 and SS2 increased in leaves and stems under a short-day regime. These results highlight the importance of photoperiod length on the transcriptional regulation of AGPase isoforms in stem and leaves.

2. Materials and methods

2.1. Plant material

Lentil (*Lens culinaris* Medik., cv. Firat87) seeds were obtained from the Southeastern Anatolia Agricultural Research Institute. The plants were grown in individual pots in a controlled climate chamber at 25 °C with supplementary humidity. Leaves, stems and roots were harvested from 24-day-old plants at 2 h intervals. Tissues were used immediately or stored at −80 °C until use. For the expression profile analysis, seeds were harvested on the 15th day after flowering and stored at −80 °C until use.

2.2. Total RNA extraction and first-strand cDNA synthesis

Tissues were homogenized in liquid nitrogen with a mortar and pestle. Approximately 100 mg of powdered tissue was used for total RNA isolation. Total RNA was extracted from leaves, stems and roots using Trizol® reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocol (phenol/guanidine isothiocyanate procedure). Total RNA was isolated from seeds using the method of Chang et al. [31] because of their high polysaccharide content. Seeds were frozen in liquid nitrogen and ground with a mortar and pestle. One ml of extraction buffer (2% CTAB, 2% PVP K30, 100 mM Tris–HCl, pH 8.0, 25 mM EDTA, pH 8.0, 0.5 g/L spermidine) including freshly added β-mercaptoethanol (2%) was used per 100 mg of powdered tissue. The samples were extracted twice with chloroform followed by the addition of ¼ vol of 10 M LiCl to the aqueous phase. RNAs were precipitated at 4 °C overnight and centrifuged at 12,000 × *g* for 20 min at 4 °C. The resulting pellets were resuspended in 100 μl of DEPC-dH_2O. The quality and quantity of the total RNA were examined by agarose gel electrophoresis and an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Deleware, USA), respectively. Total RNA (1 μg) was reverse transcribed using the First Strand cDNA Synthesis Kit™ (MBI Fermentas, Hanover, MD, Germany) with M-MLV reverse transcriptase and a random hexamer primer.

2.3. Degenerate and gene-specific primer design

Degenerate and gene-specific primers (Table S1) were designed based on the most highly conserved regions according to a sequence alignment of homologous genes in other Leguminosae (*Pisum sativum*, *Proteus vulgaris*, *Cicer arietinum*, *Vicia faba*, and *Vigna radiata*). After obtaining the desired fragment, gene-specific primers were designed. The BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast) was used to ascertain the specificity of the gene-specific primers.

2.4. PCR amplification of cDNAs

PCR was performed in a total volume of 25 μl containing 3 μl of cDNA, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 10–20 pmol of each primer, 2.5 μl of 10× buffer, and 1 U of Dream Taq DNA polymerase (MBI Fermentas, Hanover, MD). The PCR program on the icycler™ thermal cycler (BioRad Laboratories, Hercules, CA) included an initial denaturation step at 94 °C for 5 min followed by 35–45 cycles of 30 s at 94 °C, 45 s at 45 °C, 90 s at 60 °C, and a final extension step of 10 min at 60 °C. A 15-μl aliquot of the reaction was electrophoresed on a 1% standard agarose gel at 100 V for 15 min. After staining with ethidium bromide, the fragments were visualized on a UV transilluminator. PCR products of the expected sizes were gel purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia California, USA) according to the manufacturer's instructions. Approximately 100 ng of each purified PCR product was ligated into the pGEM-T Easy vector (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. Positive clones containing inserts of the correct sizes were sequenced (using T7 and SP6 primers) by the Burc Laboratory (Istanbul, Turkey). The resulting sequences were analyzed using BLASTN (http://www.ncbi.nlm.nih.gov/blast).

2.5. Phylogenetic tree analysis

The deduced amino acid sequences and alignments were analyzed using the BLAST search program (National Center for Biotechnology Information, NCBI). The conserved peptide domains were predicted using SDSC Biology Workbench/ClustalW. The phylogenetic tree was constructed using a neighbor-joining method with the MEGA4.1 program.

2.6. Quantitative real-time

Relative quantification of each gene expression was performed as follows: real time PCR was performed in a total volume of 20 μl containing 10 μl of 2X SYBR Green PCR Master Mix (TaKaRa, Japan), 10 pmol of each gene-specific primer, 1 μl of cDNA, and distilled water. The PCR protocol on the Light Cycler 1.5 (LC) (Roche Diagnostics, Mannheim, Germany) included an initial denaturation step (95 °C for 2 min) followed by amplification and quantification steps repeated for 30 cycles (95 °C for 5 s, 58 °C −60 °C for 10 s, and 72 °C for 20 s with a single fluorescence measurement at the end of the elongation step at 72 °C), a melting curve program (65–98 °C with a heating rate of 0.2 °C s^{-1} and a continuous fluorescence measurement) and a final cooling step (40 °C). Each sample was amplified with gene-specific and reference gene primer pairs. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected as an internal reference gene for normalization purposes because it is consistently expressed in all the tissues examined in this assay [32], which was also shown for *C. arietinum* L. For comparative purposes, the mean expression value of each gene at the "0 hour" time point was arbitrarily set to 1, and