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Research article

Cloning and expression analysis of transketolase gene in *Cucumis sativus* L.

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Transketolase (TK, EC 2.2.1.1) is a key enzyme in the photosynthetic carbon reduction cycle (Calvin cycle). A full-length cDNA encoding transketolase (TK, designated as *CsTK*) was isolated from cucumber leaves (*Cucumis sativa* L. cv 'Jinyou 3') by RT-PCR and RACE. The cDNA contained 2368 nucleotides with a complete open reading frame (ORF) of 2229 nucleotides, which was predicted to encode a peptide of 742 amino acids. Expression analysis by real-time PCR and western blot revealed that TK mRNA was abundant in cucumber leaves and detectable in stems, fruits and roots. TK activity and the gene expression at the mRNA and protein levels was higher in mid-position leaves (4th apical leaves) than in upper position leaves (1st) and base position leaves (12th). The diurnal variation of *CsTK* expression and TK activity in the optimal functional leaf (4th leaf) was a single-peak curve, and the peak appeared at 14:00 on a sunny day. Low temperature (5 °C) and low light (100 μmol m^{-2} s^{-1}) induced *CsTK* expression, but the expression level decreased after 24 h of chilling stress. Over-expression of *CsTK* increased the TK activity, mRNA abundance and activities of other main enzymes in Calvin cycle, and net photosynthetic rate (Pn) in transgenic cucumber leaves. Transgenic plants showed a higher ratio of female flower and yield relative to the wild type (WT) plants. The decreases in Pn and carboxylation efficiency (CE) were less in transgenic plants than that in WT during low temperature and low light intensity.

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1. Introduction

Transketolase (TK, EC 2.2.1.1) is a ubiquitously occurring enzyme that catalyzes the reversible transfer of a glycolaldehyde group from an activated ketose (donor) to the acceptor, an activated aldose [1]. The enzyme functions as a homodimer and requires thiamine pyrophosphate as a co-substrate [2]. In non-photosynthetic organisms, TK connects the pentose phosphate pathway to glycolysis to generate reducing power in the form of NADPH. In plants, TK participates in the Calvin cycle, and controls photosynthetic carbon fixation [3]. Henkes et al. [4] revealed that a small decrease of plastid TK activity in antisense tobacco transformants has dramatic effects on photosynthesis. This unexpected discovery demonstrates the central role of TK in primary metabolism. Additional results suggest that it may be possible to increase photosynthetic carbon fixation and growth by increasing the levels of TK [5]. Previously, however, TK was assumed to be a "non-regulated" enzyme in plants. Work on the characteristics of TK in yeast [6], bacteria [7], animals [8,9] and human [10] has been published, but less information has been provided for plants.

TK was first isolated from spinach by Villafranca and Axelrod in 1971 [11]. Later, it was confirmed that TK participated in photosynthetic carbon fixation in plants [12]. Since then, the enzymatic properties and biological function of TK in plants have been investigated [1,3,4]. It is known that plants contain at least one major isoform of TK [13], which is located in the chloroplast [13,14]. TK catalyzes glyceraldehyde-3-phosphate and fructose-6-phosphate or sedoheptulose-7-phosphate to xylulose-5-phosphate and erythrose-4-phosphate or ribose-5-phosphate. Either the immediate substrates or the products of the reaction are the precursors for the synthesis of nucleic acids, aromatic amino acids and vitamins [15]. In particular, xylulose-5-phosphate and ribose-5-phosphate are both essential for ribulose-1,5-bisphosphate (RuBP) regeneration.

It is well documented that TK is not induced by light [16]. However, antisense tobacco plants grown in greenhouse conditions (daily irradiance levels between 170 μmol m^{-2} s^{-1} and 600 μmol m^{-2} s^{-1}), the

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flux control value for TK over carbon fixation in ambient CO_2 and low light (170 μmol m^{-2} s^{-1}) was 0.07; this value increased to 0.32 when photosynthesis was measured at high irradiance (700 μmol m^{-2} s^{-1}) and ambient CO_2 [4]. This result indicates that the catalytic activity of TK in the Calvin cycle is regulated by light. Recently, we found that the mRNA level and the activity of TK in leaves changed markedly when cucumber seedlings were treated with suboptimal temperatures (day/night 18 °C/12 °C) and low light (100 μmol m^{-2} s^{-1}) for different days [17]. In northern China, low temperature and low light intensity are the most common abiotic stresses for cucumber plants cultivated in solar-greenhouses. These conditions often limit the growth, yield and quality of the cucumber. One of the strategies taken to overcome these limitations is engineering cucumber plants with a suitable gene that allows the plants to adapt to the special environment in a solar-greenhouse. Therefore, we isolated and characterized the TK gene (*CsTK*) from cucumber leaves grown in a solar-greenhouse. We are interested in the expression patterns of *CsTK* in different tissues and leaf positions when exposed to different light intensities and the role of the overexpression of *CsTK* in photosynthetic carbon metabolism of cucumber plants. The results of this work will be helpful in providing evidence for future research on improving the yields of cucumber and other horticultural crops.

2. Results

2.1. Cloning and sequence analysis of CsTK

cDNA was isolated from cucumber leaves. The full-length sequence of the cDNA consisted of 2368 bp nucleotides with a 2229 bp open reading frame at 25–2253 bp, encoding a 742-residue polypeptide. The deduced amino acid sequence of the cDNA showed that it encoded a polypeptide of approximately 80.62 kDa, with a calculated isoelectric point of 6.0. This cDNA was designated as *CsTK* and given the GenBank accession number JF972598. Sequence comparison showed that TK in cucumber plants contains a highly conserved thiamine diphosphate binding domain at the N-terminal which is similar to the structure of other TKs, such as *Escherichia coli*, mouse, potato, etc. [18]. Alignment of the sequences using DNAman and the NCBI's BLAST program revealed that *CsTK* has 87%, 92%, 93%, 83% and 89% similarities to the TK amino acid sequences of *Zea mays*, *Nicotiana tabacum* L., *Solanum tuberosum*, *Craterostigma plantagineum* and *Arabidopsis thaliana*, respectively (Fig. 1).

2.2. CsTK expression characteristics in wild cucumber plants

The mRNA expression level of *CsTK* in cucumber plants was investigated by real-time RT-PCR analysis. The results showed that *CsTK* was detected in leaves, stems, fruits and roots. The mRNA was more abundant in leaves than in other plant organs ($P < 0.05$) (Fig. 2A). Western blot analysis, using an antiserum against *CsTK*, revealed the presence of a strong protein signal corresponding to the levels of mRNA in leaves and weak signals in fruits, stems and roots (Fig. 2C), so leaves were used as materials in the following analysis.

Fig. 2D shows that the *CsTK* mRNA expression was higher in mid-position leaves (4th apical expanded leaves) than in upper position leaves (1st) and base position leaves (12th). A similar expression pattern was found at the protein level, with the strongest protein signals in the 4th leaves, and the weakest signals in the 8th and 12th leaves (Fig. 2F). These results indicated that *CsTK* was expressed extensively in expanded leaves from the top to the base of cucumber plants. The transcripts were relatively more abundant in mid-position leaves than in others, so mid-position leaves were used in the following investigation of the diurnal changes of *CsTK* expression.

The diurnal variation of *CsTK* expression in cucumber leaves (Fig. 3A) was a single-peak pattern on a sunny day. The level of *CsTK* mRNA increased with elevated PFD (Fig. 3C) and air temperature (Fig. 3D) in the morning and reached a maximum at 14:00. Subsequently, the level of *CsTK* mRNA decreased with a gradual decrease of PFD and air temperature in the afternoon.

The expression of *CsTK* at 5 °C for different times was measured under low light. The mRNA abundance elevated within the first 6 h, then decreased after 12 h of stress (Fig. 4A). In the leaves of treated cucumber, the strongest protein signals of *CsTK* were observed after treatment at 5 °C for 6–12 h, while a weak signal was noted after 24 h (Fig. 4C). These results indicated that low temperature and low light induced *CsTK* expression, but the expression level decreased after 24 h of low temperature and low light stress.

2.3. TK activity in wild cucumber plants

The TK activity was measured using 0.1 g samples of different position leaves, stems, fruits and roots of cucumber plants. The results showed that the TK activity in leaves was noticeably higher than the activity in stems, fruits and roots ($P < 0.05$) (Fig. 2B). TK activities were shown to be the highest in the 4th leaves, which were significantly higher than the activities in the 8th, 1st and 12th leaves ($P < 0.05$) (Fig. 2E). Similar to *CsTK* expression, a single-peak pattern of diurnal change in TK activity was found (Fig. 3B). Low temperature and low light led to a rapid elevation in TK activity during the first 6 h (Fig. 4B). Afterwards, the TK activity in treated cucumber leaves decreased gradually.

2.4. Molecular characterization of the transgenic plants

Transgenic plants infected with *Agrobacterium tumefaciens* carrying the *CsTK* gene were detected by PCR after an initial screening with 50 mg L^{-1} kanamycin. Five individual kanamycin-resistant lines were obtained from tissue culture (data not shown). These initial kanamycin-resistant plants were named T0, and the progeny obtained from T0 were named T1. Four lines named T1-2, T1-3, T1-4, and T1-5 were selected for real-time PCR and western blot analysis. Kanamycin-resistant T1 plants were checked by PCR. The upstream primer of PBI121 and the 3′ primer of *CsTK* were used in the amplification. An intense, 2368 bp band corresponding in size to the *CsTK* gene product was obtained from the kanamycin-resistant plants, whereas no band was observed for the WT plants (data not shown). Real-time PCR showed that the level of *CsTK* mRNA increased by 1.8 fold, 1.0 fold and 0.4 fold in the T1-2, T1-3, and T1-4 plants, respectively, relative to the WT plants, but no significant difference was found in the levels of *CsTK* mRNA between the T1-5 plants and the WT plants (Fig. 5A). Western blot analysis with an antiserum against *CsTK* revealed the presence of strong protein signals corresponding to *CsTK* in transgenic plant leaves, whereas a weak signal was found in WT cucumber leaves (Fig. 5C).

2.5. Activity of TK in transgenic cucumber leaves

The activity of TK in WT and T1-2, T1-3, T1-4, and T1-5 transgenic cucumber leaves was measured. Fig. 5B shows that the activity of TK in the four selected transgenic cucumber lines (T1-2, T1-3, T1-4, and T1-5) was 24%, 22%, 13%, and 15% higher than the activity in WT plants, respectively ($P < 0.05$). The results indicate that over-expression of *CsTK* increases the activity of TK in transgenic plants relative to the activity of WT plants.

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