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Isolation and characterization of two genes of the early light-induced proteins of *Camellia sinensis*

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Abstract

Early light-induced proteins (ELIPs) are nuclear-encoded thylakoid proteins. In the present research, two full-length cDNAs (741 and 815 bp), encoding ELIPs (190 and 175 aa) and their genomic sequences, were isolated from tea leaves, and named *CsELIP1* and *CsELIP2*, respectively. Both the deduced CsELIPs contain a chloroplast transit peptide in the N-terminus and a chlorophyll *a*/*b* binding protein motif with three transmembrane helices in the C-terminus. The genomic sequences of the two *CsELIPs* conform to the three-exon pattern of *ELIP* genomic sequences of other plant species. However, the identities between two CsELIPs and ACJ09655 from gymnosperm species were higher than all of ELIP-like proteins identified from other angiosperms. Expression analysis showed that the two *CsELIP* genes were significantly up-regulated when the photoinhibition occurred in tea leaves, implying that they might be involved in photoprotection.

Additional key words: chlorophyll fluorescence; gene expression; low temperature; photoinhibition; photoprotection.

Introduction

Early light-induced proteins (ELIPs), nuclear-encoded thylakoid proteins, were initially found as proteins that were transiently induced in the very early stages of the greening process in etiolated pea seedlings (Meyer and Kloppstech 1984). They are imported into chloroplasts after their synthesis in the cytoplasm as precursors, and inserted into thylakoid membranes *via* a pathway involving cpSRP (the chloroplast signal recognition particle) (Hutin *et al*. 2003). *ELIP* genes have now been identified in almost all plant species surveyed and *ELIP* family members were found to be no more than three in one species (Peng *et al*. 2008). For example, in pea and tobacco, ELIP is encoded by a single gene (Kolanus *et al*. 1987, Harari-Steinberg *et al*. 2001). Two *ELIPs* were reported in barley and *Arabidopsis* (Grimm and Kloppstech 1987, Heddad and Adamska 2000). Although seven *RcELIP* genes were identified in *Rhododendron*, it was suggested that some of them might be allelic pairs rather than separate genes (Peng *et al*. 2008). In addition, the genomic sequences of many plant *ELIP*s were found to have three-exon structure (Heddad and Adamska 2000, Bruno and Wetzel 2004).

Amino acid sequence analysis revealed that ELIPs belong to the chlorophyll (Chl) *a*/*b* binding protein (Cab) superfamily (Adamska *et al*. 1992, Hutin *et al*. 2003). Previous reports suggested that ELIPs play a photoprotective role. For instance, ELIPs were induced in plants exposed to a variety of environmental conditions, which photoinhibited photosynthetic activity (Shimosaka *et al*. 1999, Casazza *et al*. 2005). The levels of *ELIP* mRNA and its protein remained high as long as the light stress persisted, while after a return to low light conditions, both *ELIP* mRNA and ELIP protein were rapidly degraded (Adamska *et al*. 1992). The *chaos* mutant of *Arabidopsis*, which is unable to accumulate ELIPs during light stress for lacking cpSRP43 (a subunit of the cpSRP complex), is more sensitive to light stress than the wild-type. The phototolerance of the *chaos* with *ELIP* constitutive

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Abbreviations: Cab – the chlorophyll *a*/*b* binding protein; chaos – chlorophyll *a*/*b* binding protein harvesting-organelle specific; Chl – chlorophyll; cpSRP – the chloroplast signal recognition particle; CsELIP – early light-induced proteins from *Camellia sinensis*; ELIPs – early light-induced proteins; EST – expressed sequence tag; F_m – maximal fluorescence of dark-adapted state; F_v/F_m – maximum photochemical efficiency of PSII; F_0 – minimal fluorescence of dark-adapted state; LL – low light; ML – moderate light; MM – molecular mass; ORF – open reading frame; PSII – photosystem II; RACE – rapid amplification of cDNA ends; RcELIP – early light-induced proteins from *Rhododendron*; SSH – suppression subtractive hybridization.
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expression was restored to the wild-type level (Hutin *et al*. 2003). Moreover, there is a very high lutein content and a weak excitonic coupling between Chl *a* molecules in ELIPs, as compared to other Cab, which proposes that ELIPs perhaps have a protective function within the thylakoids by binding the released Chl, or an energy dissipation function during the photoinhibition (Adamska *et al*. 1999, Tzvetkova-Chevolleau *et al*. 2007). However, several recent findings raised questions about the photoprotective function of ELIPs. For example, suppression of both *ELIP1* and *ELIP2* in *Arabidopsis* did not modify the sensitivity to the photoinhibition and photooxidation or the ability to recover from light stress (Casazza *et al*. 2005, Rossini *et al*. 2006). Thus, the physiological role of *ELIPs* is not known with any certainty (Tzvetkova-Chevolleau *et al*. 2007, Rizza *et al*. 2011).

Although increasing attention has been paid to ELIPs in many plant species, little information is available in tea (*Camellia sinensis* L.), an economic woody crop, grown in different agro-climatic zones of the world. Lately, several cold-induced ESTs, encoding ELIPs were identified from tea leaves by the suppression subtractive hybridization (SSH) strategy. Of these, two cDNA fragments (FE942101 and FE942102) have been submitted to the GenBank (Wang *et al*. 2009). In this research, we reported the isolation and characterization of the full-length cDNAs and the genomic sequences of two *ELIP* genes of tea, and the correlation between the photoinhibition and *ELIP* expression in tea leaves.

Materials and methods

Plant materials and treatments: The potted biennial plants of tea (*Camellia sinensis* L. cv. Xinyangdaye) were used in this study. All tea plants (with 4–6 branches per plant) were transferred from a greenhouse (25 ± 3°C, 50–500 μmol m^{-2} s^{-1}, 12–13 h photoperiod) to a programmed incubator (25 ± 3°C, 50 μmol m^{-2} s^{-1}, 12 h photoperiod: 7:00–19:00) and housed for 7 d prior to treatment by cold and light.

To assay the effects of the irradiance and the low temperature on the gene expression and the maximal photochemical quantum yield (F_v/F_m) of photosystem (PS) II of tea leaves, ten plants were transferred to a programmed incubator for the treatment of eight combinations of the temperatures, light intensities, and photoperiods (Table 1). At 0, 3, 6, 12, 24, 36, and 48 h after the treatment, the patulous, young leaves from control and each treatment (*i.e.*, the 2/1, 4/3, 6/5, and 8/7 in Table 1) were sampled at the same time for RNA extraction, to eliminate the errors that might result from the rhythm control of *ELIP* expression. The first measurement of F_v/F_m and leaf sampling (0 h) were conducted at 9:00 h.

3' and 5' RACE: The forward primer of 3' RACE was designed according to the sequence of FE942101 and reverse primer was *SMART primer 2* (*CLONTECH*, USA). The forward primer of 5' RACE was *SMART primer 1* (*CLONTECH*, USA) and the reverse primer was designed according to the sequence of the new fragment (Table 2). Amplification was carried out using *Promega PCR Core System I* (*Promega*, USA), with a program of 2 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 51 or 55°C, 2 min at 72°C, followed by 4 min at 72°C. PCR products were cloned into *pMD19-T easy* (*TaKaRa*, China) for sequence determination.

Table 1. Different combination of the temperatures, light and photoperiod for treating materials. LL – low light (50 μmol m^{-2} s^{-1}); ML – moderate light (200 μmol m^{-2} s^{-1}).

Group	Temperature [°C]	Irradiance	Photoperiod
1	28	LL	12 h light/12 h dark
2	5	LL	12 h light/12 h dark
3	28	LL	24 h light
4	5	LL	24 h light
5	28	ML	12 h light/12 h dark
6	5	ML	12 h light/12 h dark
7	28	ML	24 h light
8	5	ML	24 h light

Isolation of the genomic sequences: The genomic DNA was extracted from young leaves of tea plants using the *Universal Genomic DNA Extraction Kit* (*version 3.0, TaKaRa*, China). The DNA fragments were amplified using the primer pairs (Table 2) designed according to the two cDNA sequences, and cloned into *pMD19-T* for sequence determination.

DNA sequencing and sequence analysis: DNA sequencing was performed at the Lianzhong Gene Shanghai Science and Technology Research Institute. Homology search of sequences in databases was conducted using the *BLAST* program against the GenBank database (http://www.ncbi.nlm.nih.gov/). Molecular mass (MM) and isoelectric point (pI) predictions for deduced ELIPs were carried out by using various tools from *Expasy* (http://www.expasy.org/tools). Chloroplast transit peptide was predicted by *CHLOROP 1.1 Server* (http://www.cbs.dtu.dk/services/ChloroP/). Chl *a*/*b*-binding protein motif was identified by *SMART* (http://smart.embl-heidelberg.de/).

Phylogenetic analysis was performed with *ClustalW* (Thompson *et al*. 1994) using the neighbour-joining method. Confidence value for the nodes was calculated using 1,000 bootstraps. Unrooted tree and relationship dendrogram were produced by using *Maximum Likelihood* of *DNAMAN* software (*version 6.0*). ELIP protein

sequences were aligned by *ClustalX* (Thompson *et al*. 1997) and subsequently adjusted manually with *GeneDoc MPC* software (*version 2.3*).

Chl fluorescence measurements: The extent of photoinhibition was determined as the F_v/F_m ratio with *Handy PEA* (*Handy Plant Efficiency Analyzer*, *Hansatech*, UK). The samples for fluorescence measurements were similar to the leaves for the extraction of RNA from the same tea plant. All fluorescence measurements were carried out after placing samples in low light (< 10 μmol(photon) m^{-2} s^{-1}) for at least 30 min. The initial fluorescence (F_0) was recorded by turning on the weak measuring light, and the maximal fluorescence (F_m) was recorded after the saturation flash [3,000 μmol(photon) m^{-2} s^{-1}]. The intervals between two measurements were 10 min. Optimum quantum efficiency of PSII was calculated as $F_v/F_m = (F_m - F_0)/F_m$. The experiment was repeated at least three times and the results were averaged.

cDNA synthesis and real-time RT PCR analysis: Total RNA for real-time RT PCR analysis was extracted from cold-, light-treated, and control tea leaves using *RNeasy Plant Mini Kit* (*Qiagen*, Germany). cDNA was synthesized from the total RNA using a *Thermoscript RT PCR system* (*Invitrogen*, USA), with oligo$(dT)_{20}$ primers, following the manufacturer's instructions. The primers for real-time PCR were designed using *Primer premier 5.0* software according to the two *CsELIPs* and α-tubulin gene of tea (gi: 52001189, it was proven that the gene expression is steady at 0–28°C in our original research and it was used as reference for mRNA abundance) (Table 2). After the amplification steps, the melting curve was determined for each primer pair at a final stage of 15 s at 95°C, 15 s at 60°C, and 15 s at 95°C to verify the presence of only one specific product. Reactions were carried out on the *ABI PRISM 7300 Real-Time PCR System* (*Applied Biosystems*, USA) according to the procedures of Wang *et al*. (2009).

Results and discussion

Isolation and characterization of two *ELIPs* of tea plant*:* In our previous work, a full-length *ELIP* cDNA (FE942102) was identified from tea leaves by SSH approach (Wang *et al*. 2009), and designated as *CsELIP1* (the gene of *Camellia sinensis* early light-induced protein 1). Putative CsELIP1 protein is 190 aa with a predicted molecular mass of 20.1 kDa and pI of 9.10. In this study, a 3' RACE was performed using the forward primer designed according to the sequence of FE942101 and *SMART primer 2* (Table 2) to obtain the full-length sequence of FE942101. As the fragment obtained by the 3' RACE was different from FE942101, a 5' RACE was performed to gain the new cDNA sequence. The forward primer of 5' RACE was *SMART primer 1* and the reverse primer was designed according to the sequence of the new fragment (Table 2). The new homolog of FE942101 and *CsELIP1* was obtained at last, and the ORF was then amplified by PCR using the primer pair designed according to the sequence. The new sequence was designated as *CsELIP2* (GenBank accession no. GQ461356), and it was 815 bp long with an ORF of 528 bp, encoding a putative polypeptide of 175 aa with a predicted molecular mass of 18.8 kDa and pI of 9.47. *CsELIP2* exhibited 98% identity to FE942101 at the nucleotide level, and 93% identity in the amino acid sequences. No significant similarity was found in their 3'-untranslational region. In addition, *CsELIP2* shared 84% identity at the nucleotide level with *CsELIP1*. Therefore, it was proposed that *CsELIP*2 is a novel member of the *ELIP* gene family of *C. sinensis*. The result also showed that there are at least three *ELIP* genes in the genome of tea plant.

The alignment of the two CsELIPs with other ELIPs from *A. thaliana* and several woody plants was conducted at the overall amino acid level. The result showed that these ELIPs shared high homology, from 58 to 91%. The highest identity (91%) was found between CsELIP1 and ACJ09655, and the lowest (58%) was between CsELIP2 and two ELIPs (ABU98944 and ABK24316) from the North American plant species. The identities of eight ELIP C-terminuses were higher than that of their

Table 2. Primer sequences for cloning and expression of the two *CsELIPs*. Q – quantification; P – primer; * – SMART primer 2; ** – SMART primer 1. The *bold fonts* show the difference between upstream primers of gDNA $_{CsELIP1}$-P and gDNA $_{CsELIP2}$-P.

Purpose	Forward primer (5'-3')	Reverse primer (5'-3')
Q-*CsELIP1*-P	ATGGCCACGCCAGCAATGCA	GCGGAGGAGTTGTAATGGGT
Q-*CsELIP2*-P	TGGAGCCCTTGTGTAGGTC	CATTACAAGGGAATTATTGCA
Q-*Tubulin*-P	CCACTCATTCCCTCCTTGAA	ATGGCTCCATCAAACCTCAG
3'RACE-P	TGGAGCCCTTGTGTAGGTC	ATCAACGCAGAGTAGTTTTTTTT*
5'RACE-P	AACGCAGAGTACGCGGG**	CATTACAAGGGAATTATTGCA
gDNA $_{CsELIP1}$-P	ATGGCCACG**C**CAGCAA**TGCAG**	CTACACAAGGGCTCCACCTT
gDNA $_{CsELIP2}$-P	CAAATGGCCACGTCAGCAAC	CTACACAAGGGCTCCACCTT

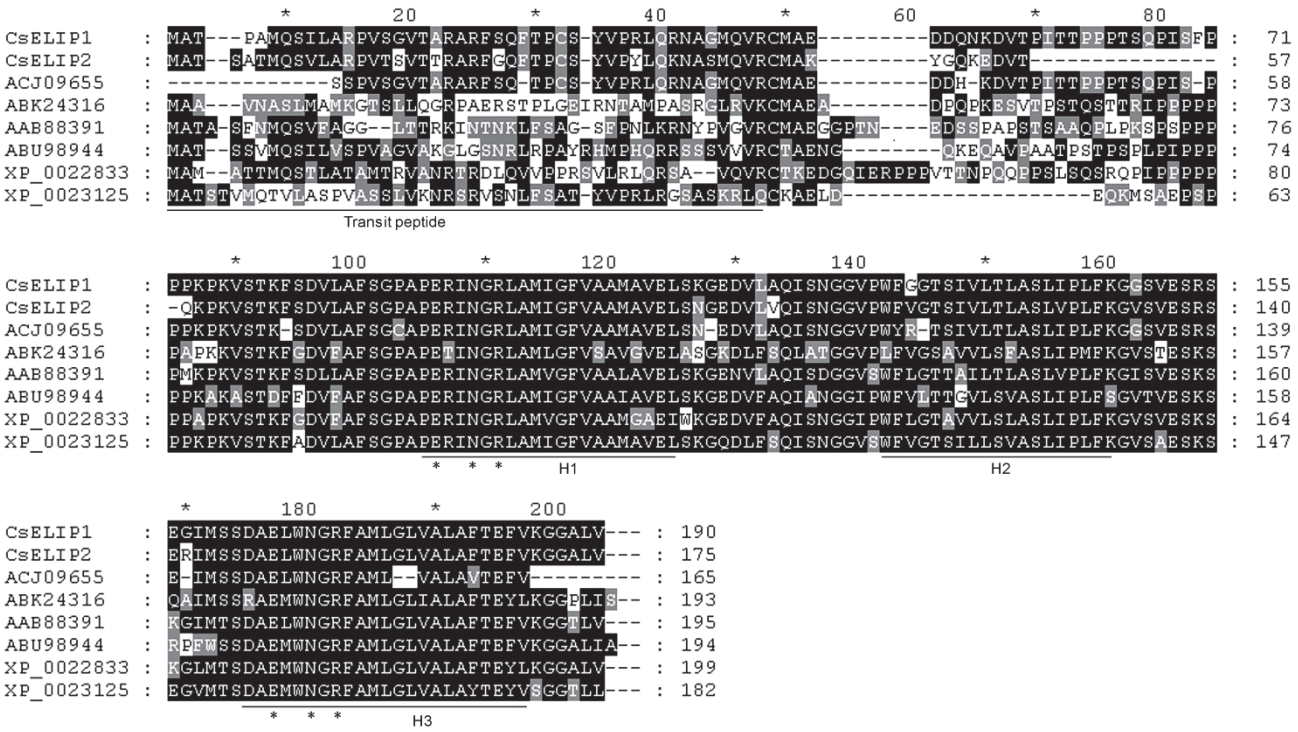

Fig. 1. Alignment of the deduced amino acid sequences of early light-induced proteins (ELIPs) from different plant species: *Camellia sinensis* (CsELIP1 and CsELIP2), *Cupressus sempervirens* (ACJ09655), *Picea sitchensis* (ABK24316), *Arabidopsis thaliana* (AAB88391), *Rhododendron catawbiense* (ABU98944), *Vitis vinifera* (XP_002283398), and *Populus trichocarpa* (XP_002312552). Identical amino acids are shown as white letters on a black background. A predicted transit peptide and three hydrophobic helices (H1, H2, and H3) are indicated by underline. The asterisks stand for the chlorophyll-binding sites in the first and third membrane-spanning helix.

N-terminuses. Three Chl-binding sites [E (Glu), N (Asn), and R (Arg)] existed separately in the first and third membrane-spanning helices of their C-terminal domains (Fig. 1) similarly as in all other known Chl *a*/*b*-binding proteins (Heddad and Adamska 2000). The high conservation of the Chl-binding sites implied that the structures might be important components of all ELIPs to perform their function. Moreover, two icosapeptide repeats, which were located in residues of 96–115 and 167–186 in CsELIP1, or 81–100 and 152–171 in CsELIP2, formed separately the bodies of the first and the third of the three helices in their C-terminal regions.

The genomic sequences (GQ461358 and GQ461359) of *CsELIP1* and *CsELIP*2 were obtained by using the primer pairs (Table 2) of amplifying the two *CsELIP* ORFs, although the downstream primers were the same due to the negligence in designing primers (Table 2). Both of them contained two AT-rich introns flanked by the consensus border sequences GT and AG. The ORF of *CsELIP1* was interrupted into three sections of 144, 84, and 345 bp in a length by two introns, which were of 129 and 855 bp, respectively. The ORF of *CsELIP2* was separated by two introns (118 and 1,202 bp) into three sections of 147, 36, and 345 bp, respectively. The three-exon pattern of the two *CsELIPs* conformed to a typical structure of *ELIP* genomic sequences of other plant species (Heddad and Adamska 2000, Bruno and Wetzel 2004).

To evaluate the structural relationship between the two CsELIPs and ELIP-like proteins from other seed-plant species, an unrooted, neighbour-joining cladogram was depicted in Fig. 2. These ELIPs could be classified into four main groups. Two CsELIPs were classified in one group, including ACJ09655 of *Cupressus sempervirens*, XP_002312552 of *Populus trichocarpa*, XP_002283398 of *Vitis vinifera*, and XP_002517068 of *Ricinus communis*. CsELIP1/CsELIP2 and ACJ09655 shared 91/74% identity at the amino acid level. However, the identities were less than 66% between the two CsELIPs and all of ELIP-like proteins, identified from other angiosperm, including *R. communis* (herb), *V. vinifera* (vine), *P. trichocarpa* (deciduous woody plant), and *R. catawbiense* (evergreen woody plant). The result suggests that different evolutionary history seemes to exist between *CsELIP*s of tea plant and the *ELIP*s known from other angiosperm species.

Effect of light and temperature on PSII photochemical efficiency of tea leaves: Chloroplast is one of the most sensitive organelle in plant cell responding to light and low temperature. When growth temperature decreased from 25 to 5°C, a considerable loss of the PSII activity has been reported in *Arabidopsis* (Montane *et al*. 1997).

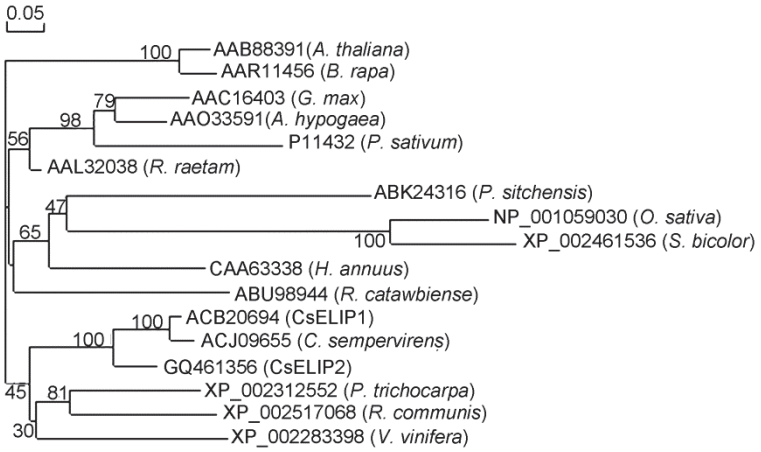

Fig. 2. Unrooted neighbour-joining tree derived from a data set of deduced amino acid sequences for *C. sinensis* ELIP1 and ELIP2 as well as other 15 ELIPs from 15 plant species: *A. thaliana* (AAB88391), *Brassica rapa* (AAR11456), *Glycine max* (AAC16403), *Arachis hypogaea* (AAO33591), *Pisum sativum* (P11432), *Retama raetam* (AAL32038), *P. sitchensis* (ABK24316), *Oryza sativa* (NP_001059030), *Sorghum bicolor* (XP_002461536), *Helianthus annuus* (CAA63338), *R. catawbiense* (ABU98944), *C. sempervirens* (ACJ09655), *P. trichocarpa* (XP_002312552), *Ricinus communis* (XP_002517068), and *V. vinifera* (XP_002283398). Numbers above the lines represent bootstrap percentages (based on 1,000 replicates).

In this study, F_v/F_m was 0.76 ± 0.01 in tea leaves under 28°C and low light (LL). F_v/F_m values were maintained at about 0.73 under combinations of 28°C and either LL or ML, with either 12-h photoperiod or continuous light. When tea plants were transferred from 28 to 5°C, the F_v/F_m declined with the increasing light intensity, and it returned to above 0.70 in dark. Under 5°C and continuous LL, the ratio of F_v/F_m remained above 0.71. These results indicated that 5°C (low temperature) alone had no obvious effect on the F_v/F_m value of tea leaves (Fig. 3*A*). However, under 5°C and ML, the ratio of F_v/F_m dropped rapidly to about 0.51 (reduced by 1/3). And under 5°C and continuous ML, F_v/F_m maintained about 0.5 at least 24 h, and then recovered slowly (Fig. 3*B*). These results showed that ML caused the mild photoinhibition in tea leaves at 5°C, but it had no such effect at 28°C. The low temperature (5°C) could cause that the light energy was excessive under such conditions.

The expression patterns of the two *CsELIP* genes: It has been reported that *ELIP* expression was induced by cold stress in other plants. However, detached leaves floated on water (Adamska *et al*. 1992, Casazza *et al*. 2005), or leaves collected in different seasons (Peng *et al*. 2008) were the plant material used in those experiments. Moreover, high light intensity (750–2,500 μmol m^{-2} s^{-1}) was used for the photoinhibition treatments (Adamska *et al*. 1992, Casazza *et al*. 2005). In the present study, the whole plants were treated under the conditions, which were similar to the changing weather in Xinyang of Henan Province of China, which is a transition region of the north and south climate, during the late autumn and the early spring. Moreover, the F_v/F_m values of the leaves were measured before the leaves were sampled for RNA extraction, in order to explore the correlation between the changes of the F_v/F_m values and the mRNA levels of two *CsELIPs*. The results indicated that the mRNA levels of the two *CsELIPs* fluctuated with the rhythm at 28°C and LL or ML, which was attributed to the presence of a circadian control-like-*ELIP* expressions of other plants (Adamska *et al*. 1991, Casazza *et al*. 2005). In our study, however, the differences between the maxima and the minima were less than 5-fold (data not shown). When tea plants were transferred from 28 to 5°C under LL and 12-h photoperiod, *CsELIP1* transcript level peaked at 3 h with a level of 17-fold of the control, and oscillated between 2-fold of control (no light) and 14-fold (in light) with circadian rhythm. Under 5°C and constant LL, *CsELIP1* mRNA level increased by 14-fold at 3 h compared with the control, it declined to about 5-fold of the control at 6 h and it was maintained to 48 h (Fig. 3*C*). If tea plants were transferred to 5°C and ML under 12 h photoperiod, *CsELIP1* mRNA abundance increased rapidly by 40-fold of the control at 3 h and descended promptly to 17-fold at 6 h, and then it fluctuated between 5- (in dark) and 40-fold (in light) of the control from 12 to 48 h. When tea plants were subjected to constant ML in 5°C, *CsELIP1* mRNA level increased promptly to 46-fold of the control within the first 3 h and descended quickly 18-fold of the control at 6 h following by a slow decline to 48 h, the oscillation characteristic of *CsELIP1* mRNA level seemed to vanish under such conditions (Fig. 3*D*).

CsELIP2 transcript level oscillated between 4-fold of control (in dark) and 10-fold of the control (in light), with circadian rhythm under 5°C, LL, and 12 h photoperiod/ constant light (Fig. 3*E*). If tea plants were transferred to 5°C and ML under 12 h photoperiod, *CsELIP2* mRNA level fluctuated vigorously between 52- (in light) and 5-fold (in dark) of the control. However, when tea plants were subjected to constant ML in 5°C, the oscillation of *CsELIP*2 mRNA level was disarranged. After increasing to 55-fold of the control at 6 h, *CsELIP*2 transcript abundance tended to decline slowly, and it was still maintained at above 30-fold at 48 h (Fig. 3*F*).

Above-mentioned results showed that the expression level of both *CsELIP* genes were maintained at a lower

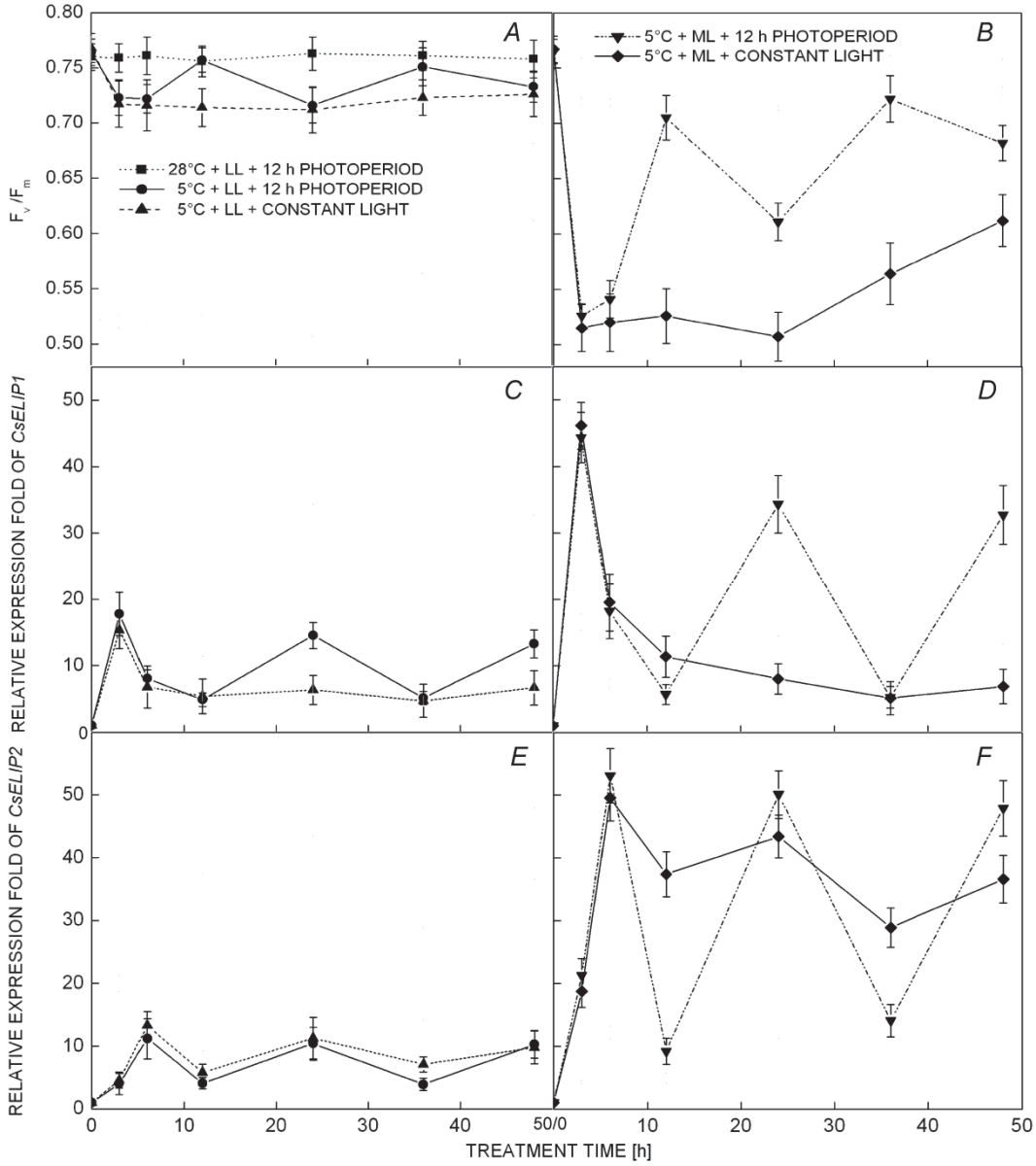

Fig. 3. The maximal photochemical efficiency of PSII (F_v/F_m) and expression analysis of two *CsELIPs* in tea leaves. (*A*) Effect of light and temperature on PSII photochemical efficiency of tea leaves under 1, 2, and 4 combination of the temperatures, light and photoperiod for treating in Table 1. (*B*) Effect of light and temperature on PSII photochemical efficiency of tea leaves under 6 and 8 treatment groups in Table 1. (*C*) *CsELIP1* expression profile in tea leaves in 2 and 4 treatment combination in Table 1. (*D*) *CsELIP1* expression profile in tea leaves in 6 and 8 treatment combination in Table 1. (*E*) *CsELIP*2 expression profile in tea leaves under 2 and 4 treatment combination in Table 1. (*F*) *CsELIP*2 expression profile in tea leaves under 6 and 8 treatment groups in Table 1. LL – low light; ML – moderate light. The data of 2 and 4 treatment combination in Table 1 were shown in order to indicate the basic expression of two *CsELIPs* under nonstress conditions. The data of 1, 3, 5, and 7 treatment combination in Table 1 were not shown as they were similar with 2 or 4 treatment combinations. The relative abundances of two *CsELIP* mRNAs both were designated as 1 in the control. The first leaf sampling (0 h) was conducted at 9:00 h. Among the sampling points during 12-h photoperiods, 12 and 36 h were in the dark. Mean ± SE, $n = 3$.

level, when the F_v/F_m values of tea leaves were maintained at above 0.7 (Fig. 3*A*,*C*,*E*); once the F_v/F_m dropped considerably (the PSII activity was restrained, Fig. 3*A*), the expression level of both *CsELIP* genes were significantly upregulated (Fig. 3*D*,*F*), suggesting that a relationship existed between the expression of two *CsELIPs* and the PSII activity. In other words, the data presented in Fig. 3 documented a time relation between the cold-dependent photoinhibition, caused by ML treatment, and the induction of two *CsELIPs* in tea leaf cells. It suggested that two *CsELIPs* had the characteristics of genes modulated by PSII efficiency in their expression

pattern (Hutin *et al*. 2003). The reduction of the photosynthetic capacity of the cells, caused by low temperature, could result in the state that the harvested light energy was relatively excessive. The excess light promotes the formation of toxic compounds, such as active oxygen species that leads to the partial inactivation of PSII complex in thylakoid membranes (Adamska *et al*. 1992, Galetskiy *et al*. 2011). Photoinhibition initiates a photoprotective response cascade (Hutin *et al*. 2003). The increase of two *CsELIPs* mRNA levels together with the increasing photoinhibition implied that the CsELIP proteins might be a photoprotective element of tea plant. It is the first report that *ELIPs* of woody plant were activated accompanying the photoinhibition.

Besides, there were some differences between the expression patterns of the two *CsELIPs*. Firstly, the response of *CsELIP1* to light stress at low temperature was earlier than that of *CsELIP2*, but the mRNA abundance and the expression duration of the former one were smaller than those of the latter. The expression of *CsELIP1* was induced only in the early period of the photoinhibition. Secondly, under the constant light, the fluctuation of *CsELIP1* mRNA level almost ceased completely with circadian rhythm, while that of *CsELIP2* was still retained (Fig. 3*C–F*). Further experiments are needed to fully understand the function and molecular mechanism of two *CsELIP* genes in the photoinhibition of tea leaves.

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