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Molecular characterization of 5-chlorophyll *a/b*-binding protein genes from *Panax ginseng* Meyer and their expression analysis during abiotic stresses

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Abstract

The chlorophyll *a/b*-binding protein (CAB) serves in both photosystems (PS), I and II, as a coordinator of antenna pigments in the light-harvesting complex (LHC). The CABs constitute abundant and important proteins in the thylakoid membrane of higher plants. In our study, five *CAB* genes, which contained full-length cDNA sequences from the 4-year-old ginseng leaves (*Panax ginseng* Meyer), were isolated and named *PgCAB*. Phylogenetic comparison of the members of the subfamily between ginseng and higher plants, including *Arabidopsis,* revealed that the putative functions of these ginseng CAB proteins were clustered into the different family of *Arabidopsis* CABs; two *PgCABs* in LHCII family and three *PgCABs* in LHCI family. The expression analysis of *PgCAB*s consistently showed dark-dependent inhibition in leaves. Expression analysis during abiotic stress identified that *PgCAB* genes responded to heavy metal, salinity, chilling, and UV stresses differently, suggesting their specific function during photosynthesis. This is the first comprehensive study of the *CAB* gene family in *P. ginseng*.

Additional key words: gene expression; gene isolation.

Introduction

Sunlight is the source of nearly all the metabolic energy driving life processes in all organisms by the photosynthetic process which converts light into chemical energy in photosynthetic organisms, such as cyanobacteria, green algae, and higher plants (Wientjes *et al.* 2013). All oxygenic photosynthetic organisms have PSI and PSII, numbered according to the historical order in which they were discovered. Excitation of PSII produces a strong oxidant capable of splitting water; operation of PSI leads to formation of a reductant that is powerful enough to reduce $NADP^+$ (Foyer and Noctor 1999). Light harvesting is the first step in the process of photosynthesis, therefore the light-harvesting antenna has to be regulated in response to physiological status and environmental signals. Chlorophyll (Chl) ligated to light-harvesting complex (LHC) proteins and carotenoids mainly serve as antenna in algae and higher plants (Green and Durnford 1996, Chitnis 2001, Gobets and van Grondelle 2001, Melkozernov 2001, Wientjes *et al.* 2013).

The evolution of the photosynthetic machineries is closely connected to the extended LHC protein superfamily. The LHC protein superfamily comprises several families, including LHC protein, LHC-like protein, the red lineage CAB-like protein, and the S subunit of PSII (PSBS) protein family (Engelken *et al.* 2010, 2012). The LHC protein family is divided into the subfamilies of the Chl *a*-binding (CAA) proteins, the Chl *a/b*-binding (CAB) proteins, the Chl *a*/*c*-binding (CAC) proteins, and the lesser known LHC clades including LHCx and LHCz. Higher plants possesses only CAB, therefore CAB is regarded as LHC, light-harvesting Chl *a*/*b*-binding protein (Jansson 1992, 1999; Tao *et al.* 2011). CAB is the most abundant membrane protein in nature (Bassi *et al.* 1997, Jansson 1999) and encoded by nuclear genes, synthesized on cytoplasmic ribosomes, imported across the two membranes of the chloroplast envelope, and finally inserted into the thylakoid membrane, in contrast to a number of chloroplast-encoded hydrophobic proteins in

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Abbreviations: CAB – chlorophyll *a/b*-binding protein; Chl – chlorophyll; EST – expressed sequence tags; MS – Murashige and Skoog; ROS – reactive oxygen species; Pg – *Panax ginseng*; At – *Arabidopsis thaliana*; Rc – *Ricinus communis*; Pv – *Phaseolus vulgaris*.

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PSI and PSII cores (Green *et al.* 1991). CAB proteins associated with PSI are named Lhca (or LHCI), and the ones associated to PSII are named Lhcb (or LHCII) (Engelken *et al.* 2012). Lhcb is the major CAB and accounts about 50% of the total Chl in the plants, indicating its importance. It is involved in both short- and long-term adaptations to different light and temperature conditions.

Since the identification of *Lhc* gene in pea (*Pisum sativum*) (Bedbrook *et al.* 1980), the CAB proteins have been identified in higher plants and distinct types of LHC were recognized. In *Arabidopsis thaliana*, ten CAB proteins are encoded in its genome, four of them are associated with PSI (Lhca1–Lhca4) and six with PSII (Lhcb1–Lhcb6) (Jansson 1999, 2006). In tomato (*Lycopersicum esculentum*), 16 CAB proteins have been isolated and/or characterized, six of them are associated with PSI (Lhca1–Lhca4), and ten with PSII (Lhcb1–Lhcb6) (Pichersky *et al.* 1985, 1987, 1988, 1989, 1991; Hoffman *et al.* 1987, Schwartz *et al.* 1991, Schwartz and Pichersky 1990). In tobacco (*Nicotiana tabacum* L.), two CAB proteins have been isolated and/or characterized, one of them associated with PSI (Lhca1), and the other with PSII (Lhcb1) (Palomares *et al.* 1991). In spinach (*Spinacia oleraceae* L.), four CAB proteins have been isolated and/or characterized, and all are associated with PSII (Lhcb1, Lhcb4, Lhcb6) (Henrysson *et al.* 1989, Spangfort *et al.* 1990).

Panax ginseng Meyer is a perennial herb in the family Araliaceae and is cultivated for its highly valued root used for medicinal purposes. Previously, we have reported one *CAB* gene (In *et al.* 2005), but no other report on *CAB* gene in ginseng has been published, despite the importance of the light sensitivity of ginseng (Harding 1908, Parmenter *et al.* 2000, In *et al.* 2010). Ginseng needs to be cultivated under special conditions to meet its requirements of about 30% full sunlight (Kim *et al.* 2014a), growing well only in shadow under canopy or under artificial shade structures (Kim *et al.* 2015). In this study, we identified five CAB proteins encoded in *P. ginseng*, two are associated with PSII (Lhcb2 and Lhcb5) and three with PSI (Lhca1 and Lhcb4). The present study examined the phylogenetic relationship of ginseng *CAB* and *Arabidopsis Lhc* genes and their relatives in order to contribute to the understanding of the possible role of ginseng CAB.

Materials and methods

Nucleotide sequencing and sequence analysis: In order to identify genes from the previously constructed expressed sequence tags (EST) libraries from four-year-old ginseng leaf (Kim *et al.* 2006), homologous sequences of *CAB* EST were searched against the *GenBank* databases using a *BLASTX* algorithm. A pTriplEx phagemid for *CAB* cDNA was excised from the λpTriplEx2 and used as a template for sequence analysis. Nucleotide and amino acid sequence analyses were performed using the *DNASIS* program (*Hitachi*, Japan).

These deduced amino acid sequences were utilized to search for homologous proteins *via BLAST* network services at the NCBI. *ClustalX* with default gap penalties was used to perform multiple alignments of *CAB*s isolated in ginseng and previously registered in other species. A phylogenetic tree was constructed by the neighbor-joining method, and the reliability of each node was established by bootstrap methods using *MEGA4* software. Secondary structures were analyzed by Self-Optimized Prediction from Multiple Alignment (SOPMA) (Geourjon and Deléage 1995). The protein properties were estimated using *ProtParam* (Gasteiger *et al.* 2005) and the hydropathy value was calculated by the method described by Kyte and Doolittle (1982). Identification of conserved motifs within *CAB* was predicted by Multiple EM for Motif Elicitation (MEME) (Bailey *et al.* 2009). The subcellular localization for N-terminal signal was predicted by iPSORT (Bannai *et al.* 2002). A three-dimensional (3-D) model was prepared using CAB as a template on a SWISS-MODEL Workspace in automated mode (Arnold *et al.* 2006). The generated 3-D structure was visualized using the UCSF *Chimera* package.

Plants and application of stress conditions: *P. ginseng* cv. "Hwang-Sook" seeds (provided by Ginseng Bank) were used. Three-week-old plantlets were used for treatments and nucleic acid extractions, as described by Kim *et al.* (2008). For chemical stress, the plantlets were placed for various periods in Murashige and Skoog (MS) media containing: 50 µM $CuSO_4$, 20% sucrose, or 100 mM NaCl. Chilling stress was applied by exposing the plantlets to 4°C. For the UV treatment, the plantlets were irradiated under UV-C lamps at 1.35 µE m^{-2} s^{-1} (below 280 nm). In all cases, stress treatments were carried out in the MS media and ten plantlets were treated with each stress for 1, 4, 24, and 48 h. Control plants were held in a growth room at 25°C under a 16-h photoperiod. Light condition for control and treated plants was 40 µmol(photon) $m^{-2}s^{-1}$. The plantlets from all completed treatments were immediately frozen in liquid nitrogen and stored at –70°C until required.

Three-year-old ginseng plants, grown hydroponically in a controlled greenhouse, were used for a dark treatment. Control plants were grown under a regime of 16 h of light and 8 h of dark and sampled under light conditions, whereas the dark treatment (covered with a black box) lasted for 2 and 3 d (Kim *et al.* 2014b).

Real-time quantitative RT-PCR: Total RNA was extracted from seedlings of *P. ginseng* using an RNeasy mini kit (*Qiagen*, Valencia, CA, USA). For RT-PCR, 200 ng of total RNA was used as a template for reverse transcription using oligo(dT)$_{15}$ primer (0.2 mM) and AMV reverse transcriptase (10 U μl^{-1}) (*INTRON Biotechnology, Inc.*, South Korea) according to the manufacturer's instructions. Real-time quantitative PCR was performed using 100 ng of cDNA in a 10-μl reaction volume using *SYBR® Green Sensimix Plus Master Mix* (*Quantace,* Watford, England). Specific primers for each of *PgCAB*s were used to perform real-time PCR (Table 1S, *supplement available online*). A constitutively expressed β-actin gene with primer (forward) 5'-CGT GAT CTT ACA GAT AGC TTG ATG A-3′ and (reverse) 5'-AGA GAA GCT AAG ATT GAT CCT CC-3' was used as internal reference. The thermal cycler conditions recommended by the manufacturer were used as follows: 10 min at 95°C; followed 40 cycles of 95°C for 10 s; 60°C for 10 s; and 72°C for 20 s. The fluorescent product was detected at the last step of each cycle. Amplification, detection, and data analysis were carried out with a *Rotor-Gene 6000* real-time rotary analyzer (*Corbett Life Science*, Sydney, Australia). Threshold cycle (Ct) represents the number of cycles at which the fluorescence intensity was significantly higher than the background fluorescence at the initial exponential phase of PCR amplification. To determine the relative fold differences in template abundance for each sample, the Ct value for *PgCAB*s was normalized to the Ct value for β-actin and calculated relative to a calibrator using the formula $2^{-\Delta\Delta Ct}$. Three independent biological replicates were performed to qRT-PCR runs in triplicate. PCR products were characterized by the melting curve analysis. The primer efficiencies were determined according to the method of Livak and Schmittgen (2001) to validate the ΔΔCt method used in our experiment. The observed slopes were close to zero, indicating that the efficiencies of the gene and the internal control β-actin were equal.

Statistical analysis: Means of three independent replicates were statistically analyzed and compared with control (at $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) using *Student*'s *t*-test.

Results

Isolation and sequence analysis of five PgCABs: From our EST library, that was previously constructed from leaf of four-year-old *P. ginseng* (Kim *et al.* 2006), we identified five cDNA clones encoding the *CAB* gene. We named these genes *PgCAB1* to *PgCAB5* (*P. ginseng* Chl *a/b*-binding protein). The corresponding characteristics of each *PgCAB*s are indicated in Table 1. The full-length cDNA sequences were assigned to GenBank under the accession numbers (KP874095-KP874099). In addition, the tertiary structure of matured proteins without transit peptides were observed by 3-D modeling (Fig. 1). PgCAB1, PgCAB3, PgCAB4, and PgCAB5 were composed of three α helixes, which are involved in the interaction with a closed pigment, resulting in formation of dimers (Külbrandt *et al.* 1994, Melkozernov and Blankenship 2004). The motif 2, which is mostly conserved within the PgCABs was also identified in each model that contains the conserved LHC motif.

Table 1. Characteristics of ginseng CABs. Length (number of amino acid residues), molecular mass, and isoelectric point (pI) of PgCAB proteins deduced from the open reading frames for mature protein.

Protein	Length [amino acids]	Molecular mass [kDa]	pI
PgCAB1	239	25.82	5.13
PgCAB2	262	28.08	5.51
PgCAB3	222	24.49	6.08
PgCAB4	222	24.49	5.52
PgCAB5	218	23.73	5.59

Homology analysis: A *GenBank BlastX* search revealed that the deduced amino acid sequences of PgCAB1 share higher degrees of identity (99 and 97%) with the CAB protein of *P. ginseng* (BAE4638) and *Aralia elata* (AF067217), respectively. PgCAB2 shares the highest degrees of identity (87%) with the CAB protein of *Phaseolus vulgaris* (AGV54683.1). PgCAB3 shares the highest degrees of identity (86%) with the CAB protein of *Beta vulgaris* (CAE30280). PgCAB4 shares the highest degrees of identity (88%) with the CAB protein of *Phaseolus vulgaris* (AGV54882) and PgCAB5 shares the highest degrees of identity (86%) with the CAB protein of *Ricinus communis* (EEF29136).

Since the complete set of *CAB* genes has been characterized from *Arabidopsis*, the full-length protein sequences of CAB isozymes and their relatives in higher plants including *A. thaliana* (Jansson 1999) were used to construct a phylogenetic tree (Fig. 2*A*). The resulting tree generated two groups, PSI and PSII, and four distinct branches for antenna proteins corresponding to: LHCII type 2, LHCII type 1, CP26 protein, and LHCI-730 protein. The five PgCABs were clustered into three related proteins. PgCAB1 and previously reported PgCAB (In *et al.* 2006) belong to the LHCII type 2 protein family as isozymes*,* PgCAB2 belongs to the CP26 protein family, and PgCAB3, PgCAB4, and PgCAB5 belong to LHCI-730 protein family. These results undoubtedly provide important clues for studying the function of ginseng *CAB* genes. In addition, conserved motifs were found by MEME analysis in all plant CAB isozymes (Fig. 2*B*). There are

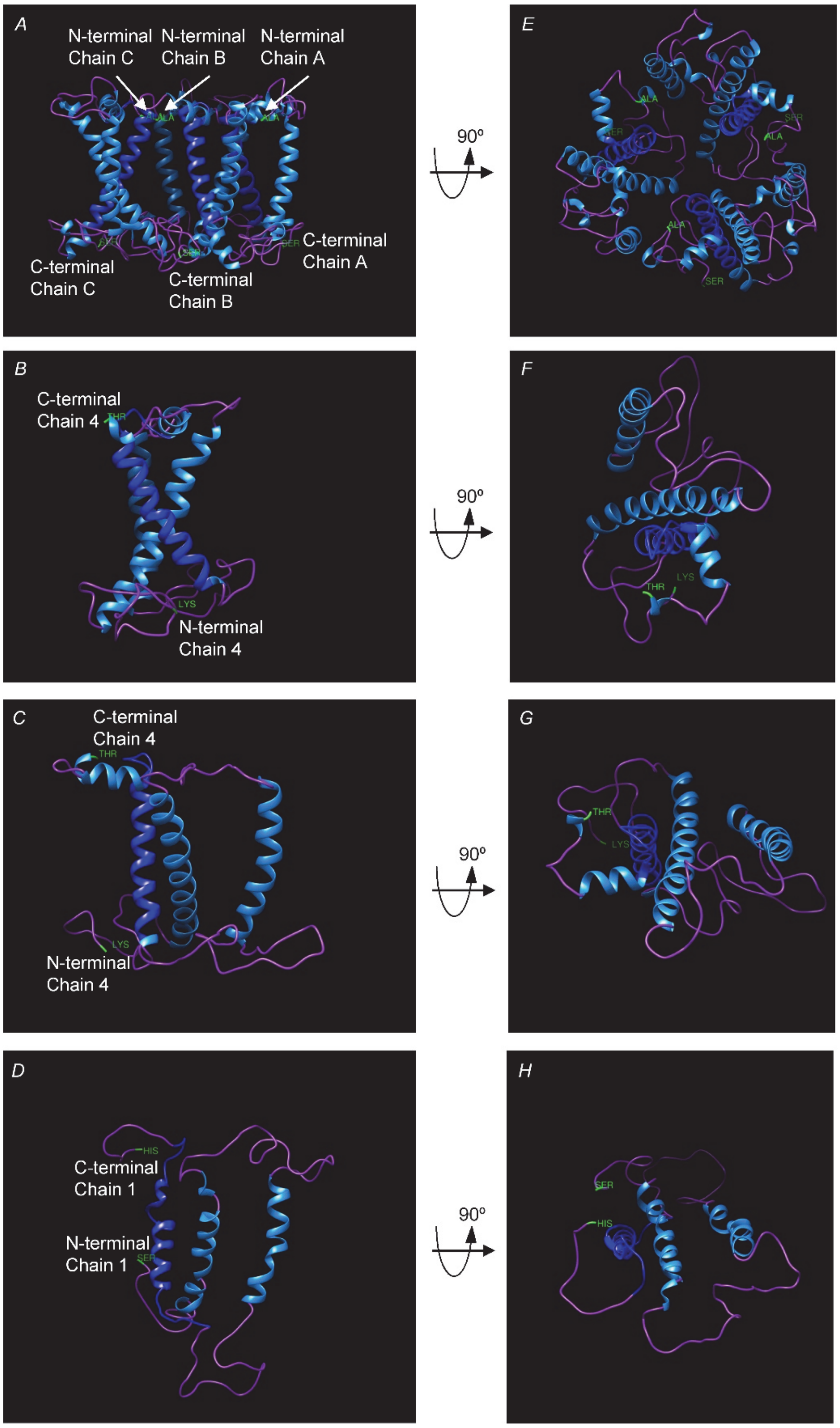

Fig. 1. The predicted 3-D structures of PgCABs. Comparative representation was performed by UCSF *Chimera* package and helix and coil structures are depicted as sky-blue and purple, respectively. Motif 2 protein sequences analyzed by MEME are depicted as blue. The top view of *A*: PgCAB1, *B*: PgCAB3, *C*: PgCAB4, and *D*: PgCAB5 are shown in *E* to *H*, respectively.

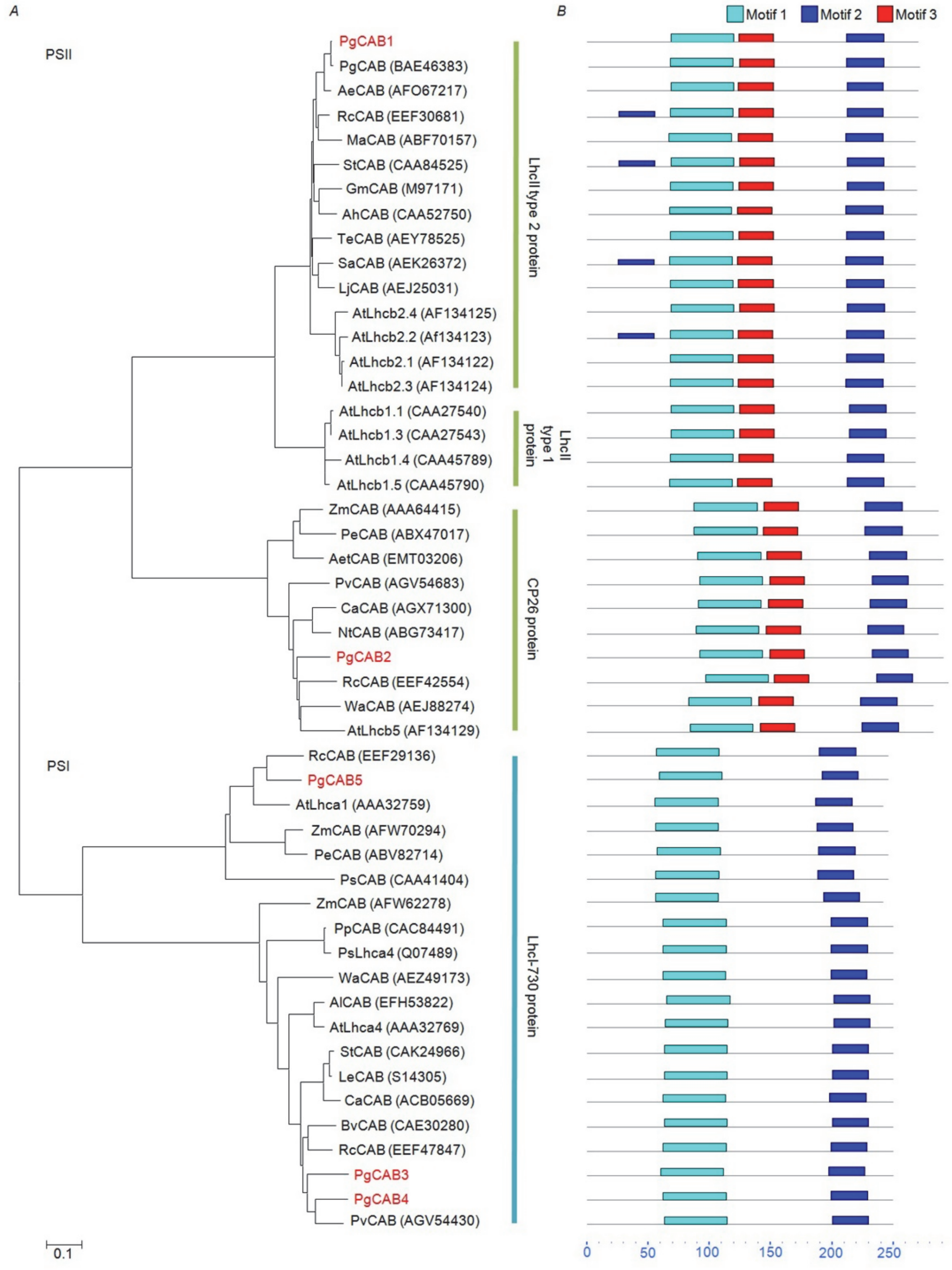

Fig. 2. *A*: Sequence homology analysis of PgCABs with LHC proteins associated with *Arabidopsis* and higher plants. A phylogenetic tree of PgCAB1, PgCAB2, PgCAB3, PgCAB4, and PgCAB5 (in *bold red letter*). The neighbor-joining method was used, and the branch lengths are proportional to the divergence, with the scale of 0.1 representing 10% changes. Protein sequences were from the databases indicated in parentheses. *B*: Organization of putative motifs in CAB identified by MEME. Numbered color boxes represent different putative motifs, and the sequences of the motifs are listed in supplementary Fig. 2S (*available online*). Motifs 1, 2, and 3 are indicated by the mint, blue, and red boxes, respectively. Motif sizes are indicated at the bottom of the figure.

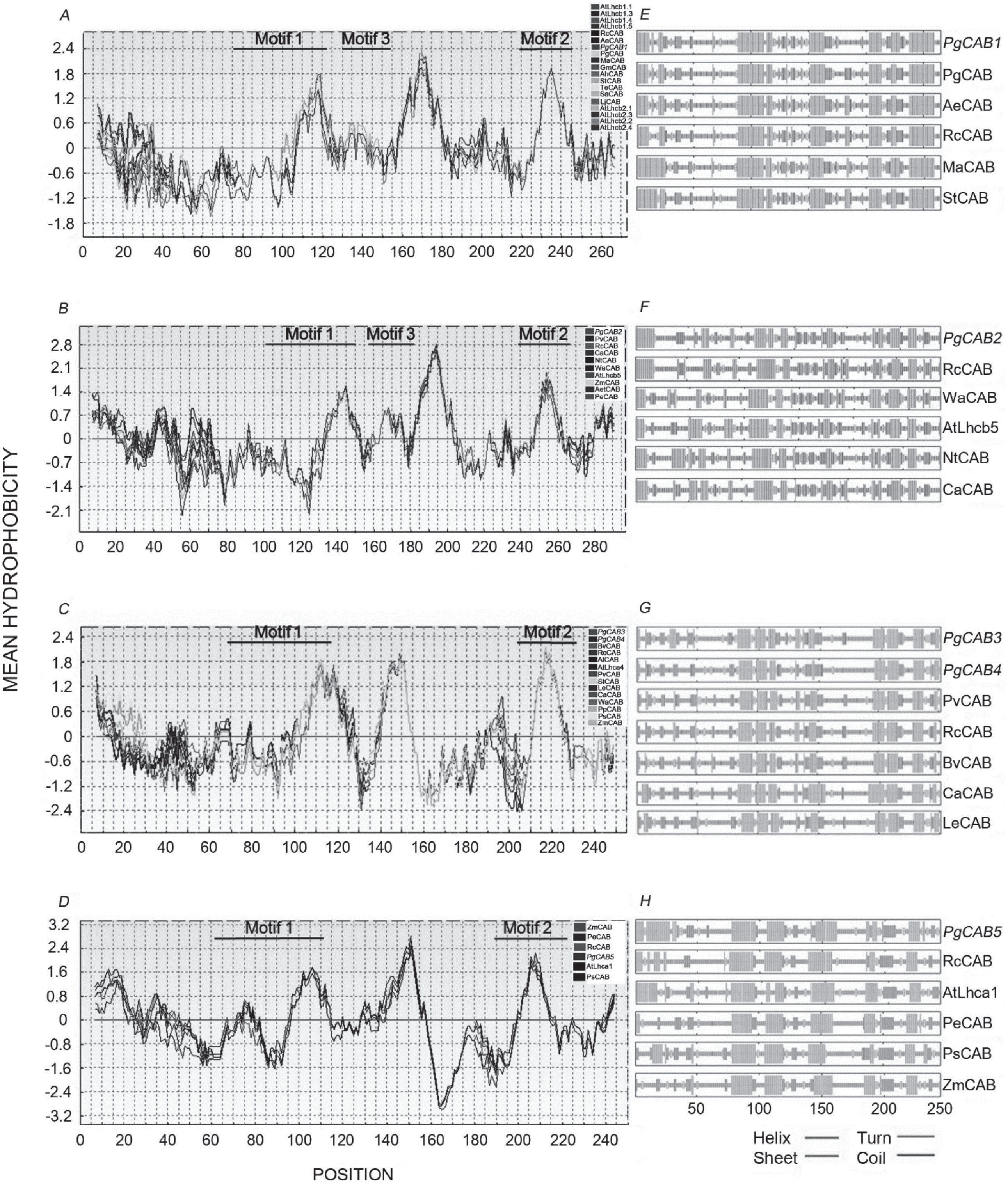

Fig. 3. Superimposed hydrophobicity profiles and secondary structure predictions for each PgCAB group and homologous. Hydrophobic domains are indicated by positive numbers, hydrophilic domains are above the line and hydrophilic domains are below the line. *A*: PgCAB1 group associated with AtLhcb2, *B*: PgCAB2 group associated with AtLhcb5. *C*: PgCAB3, PgCAB4 group associated with AtLhca4, and *D*: PgCAB5 group associated with AtLhca1. (*E*, *F*, *G*, *H*): Comparison of CAB secondary structures by SOPMA. The helix, sheet, turn, and coil are indicated in the order from the longest to the shortest.

two motifs in the PgCABs associated to the PSI group (PgCAB3, PgCAB4, PgCAB5), and three conserved motifs were found in PgCABs associated to the PSII group (PgCAB1, PgCAB2), indicating that these two motifs are highly conserved in all analyzed CAB sequences and PSI-dependent motif 3. All groups of CAB proteins share significant sequence conservation 'ELINGRLAMLGFLG FLVPELIT' (called 'LHC motif), a highly hydrophobic sequences (including Glu, Arg, and Gly residues) responsible for the core complex (Jansson 1999), within the motif 2 region (Fig. 1S*A–D*, *supplement available online*).

The similarity of the hydrophobicity profile of the estimated CAB protein of the four groups with the *Lhc* relative genes in *Arabidopsis* (Jansson 1999) is shown in Fig. 3*A–D*. It revealed that the N-terminal was very different in each group. The vast majority of stromal and thylakoid proteins are imported by a common default pathway in which the imported protein is synthesized with a cleaved N-terminal presequence, often termed 'the transit peptide', and import is mediated by the concerted action of protein translocation systems in the outer and inner envelope membranes post-translationally (Green *et al.* 1991, Jensen *et al.* 2007). In contrast, the C-terminal was highly conserved, and it has been reported to be involved in the stabilization of trimeric LHC (Kuttkat *et al.* 1996).

A model from the chloroplast photosynthetic apparatus of the PgCABs characterized in this study is presented in Fig. 3S (*available online*), adapted from Allen *et al.* (2011).

The secondary structure analysis, conducted by SOPMA, revealed high similarity of PgCABs to the secondary structure of other plant CABs with close phylogenetic relationship (Table 2), showing similar number of alpha-helices, beta-turns, extended strands, and random coils.

***PgCAB* genes were differentially expressed in diverse organs**: In order to examine the expression profiles of the *PgCAB* genes in different ginseng tissues, real-time PCR was carried out using the cDNA templates from three organs, including leaf, stem, and roots. *PgCAB1*, *PgCAB2*, *PgCAB3*, and *PgCAB5* showed similar expression pattern in leaves, stem, and roots, whereas *PgCAB4* showed that leaf and stem expression level was equal, and lower expression was observed in roots (Fig. 4). Under natural conditions, the five *PgCAB* genes were relatively highly expressed in all tested ginseng tissues showing preferential expression in the leaves.

Temporal expression of *PgCAB* genes in response to abiotic stresses: The expression patterns of the *PgCAB* genes at different time points after treatments with different abiotic stimuli were analyzed using real-time PCR (Fig. 5). In contrast to the enhanced expression under light, the dark condition caused a significant decrease of *PgCABs* (Fig. 5*A*). As a result of the copper stress (Fig. 5*B*), *PgCABs* expression was reduced compared with control. The NaCl stress (Fig. 5*C*) caused that both *PgCAB1* and *PgCAB2* were significantly upregulated after 24 and 48 h of the treatment. *PgCAB3* and *PgCAB4* significantly increased after 24 h and decreased after 48 h of the treatment, while *PgCAB5* significantly decreased compared with control.

Table 2. Secondary structure characteristics of ginseng CABs and other plants with close phylogenetic relationship.

Protein	Alfa-helices	Beta-turns	Extended strands	Random coils
PgCAB1	103	33	43	86
PgCAB (BAE46383)	102	31	42	90
PgCAB2	87	31	57	113
RcCAB (EEF42554)	98	28	50	116
PgCAB3	79	25	47	97
PgCAB4	64	27	46	113
PvCAB (AGV54430)	72	21	47	112
PgCAB5	100	23	36	85
AtLhca1 (AAA32759)	90	24	31	86

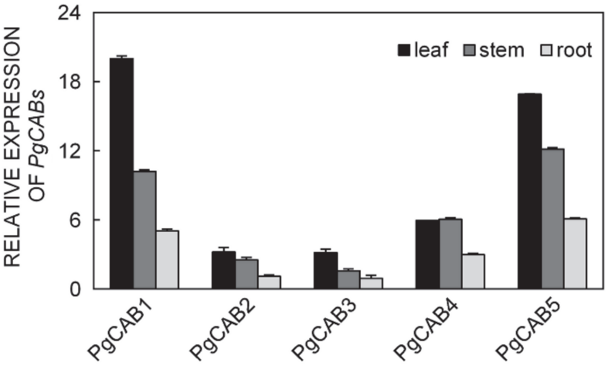

Fig. 4. Expression of *PgCAB* genes in leaves, stem, and roots of three-year-old *Panax ginseng*. Bars indicate the mean value ± SE from three independent experiments.

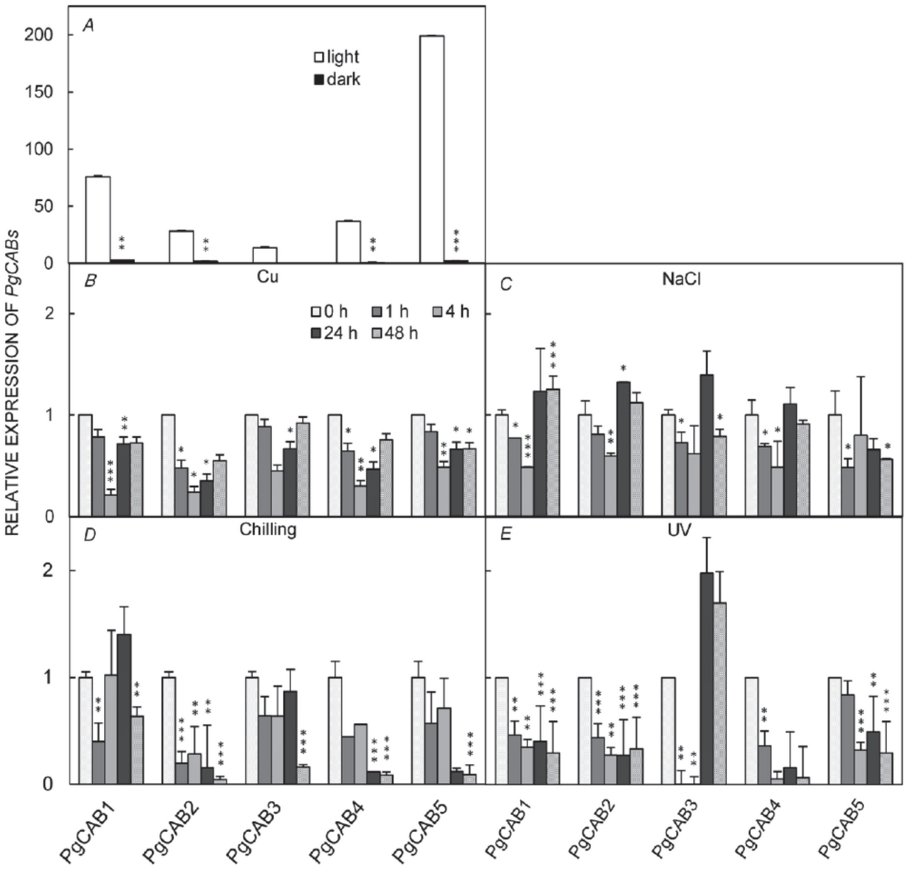

Fig. 5. Relative quantities of *PgCAB1, PgCAB2, PgCAB3, PgCAB4,* and *PgCAB5* mRNA at various time points of post-treatments with various stresses: *A*: light-dark, *B*: 100 mM $CuSO_4$, *C*: 100 mM NaCl, *D*: chilling, *E*: UV light. Bars indicate the mean value ± SE from three independent experiments. 0 h sample was used as calibrator. Means of three independent replicates were statistically analyzed and compared with control (at $*p<0.05$, $**p<0.01$, $***p<0.001$) using *Student*'s *t*-test.

Following the chilling stress (Fig. 5*D*), *PgCAB1* gene expression increased to the highest expression level at 24 h and decreased significantly 48 h after the treatment, while the *PgCAB3* gene expression increased to its highest expression level at 24 h and decreased significantly at 48 h after treatment. In case of *PgCAB2*, *PgCAB4*, and *PgCAB5*, gene expressions were extremely significantly decreased at 48 h after treatment compared to control. Following the UV stress (Fig. 5*E*), *PgCAB3* was upregulated twice at 24 and 48 h after the treatment, whereas the other *PgCABs* gene expressions very significantly declined at 48 h after the treatment compared with control.

Discussion

Photosynthesis is dependent on the light harvesting by CAB-bound chlorophylls, which make up the light-harvesting antenna. *P. ginseng* is grown in shadow under a canopy, it requires low irradiance, while high light can damage ginseng leaves easily (Kim *et al.* 2006). Interestingly, Kim *et al.* (2006) reported much higher abundance of CAB in ginseng leaf cDNA library compared to other plants, such as stevia and rice ESTs, indicating important and unique expression pattern of CABs in ginseng leaves. However, only one PgCAB was reported previously (In *et al.* 2005).

The PSI and PSII represent the two basic types of photosynthetic reaction centers (Nield *et al.* 2004), and both photosystems cooperate in gathering light energy aimed at a photosynthesis-dependent carbon fixation (Wollman 2001). The CAB proteins serve to maximize and regulate light harvesting (Klimmek *et al.* 2006). PSII is composed of a core complex, where the primary photochemistry takes place, and a peripheral antenna system, encoded by *Lhcb1 – 6* genes (Jansson 1999). The major antenna of PSII is a trimeric LHC composed of a combination of *Lhcb1 – 3* gene products. The minor Lhcbs consist of three monomers, Lhcb4 – 6, also named CP29, CP26, and CP24 (Wientjes *et al.* 2013). In order to obtain an overall picture of the five ginseng CABs and their relationships with those of *Arabidopsis* and higher plants, a phylogenetic tree was constructed. It generated two groups (Fig. 2), PSI and PSII, four distinct branches for antenna proteins, LHCII type 2, LHCII type 1, CP26 protein, and LHCI-730 protein and divided the five PgCABs into three related proteins: two are associated with PSII (PgCAB1-2) and three with PSI (PgCAB3-5). The exact number of *CAB* genes in ginseng could be determined when all ginseng CAB genes are isolated from its genome. Higher identity (> 80%) of each group of CAB, rather less similarity among *PgCABs* (24–39% identity) except *PgCAB3* and *PgCAB4* (85%), supports a much later gene duplication event and very early stages of gene family evolution in eukaryotic photosynthetic organism (Green *et al.* 1991). In addition, more similarity of each PgCAB with the CABs in perennial plants, rather than *Arabidopsis*, implies its important evolutionary relationship.

PgCABs were clustered with *Arabidopsis* members: PgCAB1 with AtLhcb2 (LHCII type 2 family), PgCAB2 with AtLhcb5 (LHCII CP26 protein family), PgCAB3 and PgCAB4 with AtLhca4 (LHCI-730 protein family), and PgCAB5 with AtLhca5 (LHCI-730 protein family), providing valuable information for studying the functions of ginseng CABs. In *Arabidopsis*, *Lhca1* and *Lhca4* genes encode the polypeptides of LHCI associated with PSI. *Lhcb2* genes encode the polypeptides of trimeric LHCII, with a dual function as antenna for both photosystems and regulating the dissipation of excitation energy in excess (Horton *et al*. 2005, Ruban *et al*. 2007). *Lhcb5*, also called CP26, is probably monomeric protein that is present in one copy per PSII unit (Jansson 1999). The occurrence of 'generic LHC motif' at the C-terminus of both PSI and PSII CABs (indicated by asterisks in Fig. 1S*A*–*D*) suggests its conserved role during the evolution of the LHC proteins in higher plants and its importance for helix-helix interaction by the Arg-Glu residues (Jansson 1999). The transit peptide at N-terminal sequences of CAB is not conserved (Fischer *et al.* 1999).

It has been reported that expression of Lhcb occurs exclusively in different green tissues grown in the light, being detectable even in roots, except of dry seeds (Matsuoka 1990, Xu *et al*. 2012). The high expression of *PgCAB*s in leaves is consistent with the localization of the photosynthetic apparatus. The five *PgCAB*s were highly expressed in leaves. *PgCAB1* showed the highest intensity of relative expression, followed by *PgCAB5*, *PgCAB4*, *PgCAB2*, and *PgCAB3*. *Arabidopsis* Lhcs show large differences in their expression levels. LHCII type 1, particularly Lhcb1.3, is expressed at the highest level, whereas LHCII type 2 family and Lhcb4.3, Lhca5–6 are expressed at low levels (Janssen 1999). The higher expression level of *PgCAB1* compared to other *PgCAB*s in ginseng leaves is different from the pattern of homologous AtLhcb2, which shows low expression (Jansson 1999), suggesting their different transcriptional regulation in each species in spite of conserved sequences. In spite of expression in leaves, there is evidence that *CAB* genes are expressed also in tissues other than green. Klimmek *et al.* (2006) reported the tissue expression of poplar (*Populus spp.*) in leaves, stem, and roots. Stahl *et al.* (2004) reported the expression of CAB in sugar beet (*Beta vulgaris* L.) both in leaves and roots. Basset *et al.* (2007) reported the expression of *CAB* in leaves and roots of peach (*Prunus persica* L.). Our results confirmed that the five *PgCAB*s were also expressed in the stem and roots (Fig. 4). However, *PgCAB* genes are expressed not only in green tissues; it would be appropriate to discuss how distinct regulation patterns might reflect the expression of *PgCABs* in different tissues. There are two possible explanations for this finding. The tissue samples included a few chloroplast-containing cells or some *Lhc* genes are also expressed at very low levels in cells lacking chloroplasts (Klimmek *et al.* 2006).

The highest expression of *PgCAB5* in the three-year-old mature leaves (Fig. 4), showing difference from the expression pattern in the four-week-old leaves, could be explained by regulation during development (Bassett *et al.* 2003, Xu *et al.* 2012). It is well established that light is the most important environmental signal regulating *Lhc* expression (Silverthorne and Tobin 1984, Sun and Tobin 1990, Millar and Kay 1996, Peer *et al.* 1996, Weatherwax *et al.* 1996, Yang *et al.* 1998, Humbeck and Krupinska 2003, Staneloni *et al.* 2008, de Montaigu *et al.* 2010, Pruneda-Paz and Kay 2010, Thines and Harmon 2011). Corresponding with dark-dependent inhibition of *CAB* expression (Matsuoka 1990), the five *PgCAB*s showed very low expression under dark conditions (Fig. 5*A*), confirming the light-dependent expression of all *PgCAB* genes in leaves.

Previous studies have indicated that several environmental stresses affect *CAB*s' expression (Capel *et al.* 1998, Nott *et al.* 2006, Staneloni *et al.* 2008). Chloroplasts are major sites of reactive oxygen species (ROS) production (Kwak *et al.* 2006, Nott *et al.* 2006, Galvez-Valdivieso and Mullineaux 2010), where LHC plays a key role (Xu *et al.* 2012). The photosynthetic apparatus of higher plants is regulated in response to environmental and metabolic conditions (Bergantino *et al.* 1995). The environmental stresses, such as salinity (Munns and Termaat 1986, Munns 2005), metal ions (Larbi *et al.* 2006, Krasensky and Jonak 2012), and ultraviolet radiation (Greenberg *et al.* 1989, Joshi *et al.* 1994, 1997, 2011; Jordan 1996, 2002; Vass *et al.* 2002) have been demonstrated to act primarily at the level of the photochemical reactions (Joshi *et al.* 2013). However, functional studies of this gene family in ginseng are lacking. *PgCAB*s exhibited different expression patterns under stress treatments. Under copper stress, *PgCABs* were reduced compared with control. In case of NaCl stress, *PgCAB1*, *PgCAB2*, *PgCAB3*, and *PgCAB4* were enhanced after 24 h of the treatment, except *PgCAB1* and *PgCAB2*, both belonging to PSII, which were upregulated after 48 h compared with control. Under chilling stress, only *PgCAB1* was enhanced after 24 h of the treatment compared with control. The UV stress upregulated only *PgCAB3* compared with control. *PgCAB*s were clustered with *Arabidopsis* members, suggesting that they may have similar functions to the homologous proteins in abiotic stress regulation.

The relative expression of the five *PgCAB*s was inhibited under copper and salt stresses (Fig. 5*A*). This is consistent with the general mechanism of copper toxicity-induced inhibition of photosynthetic light reactions in bean (*Phaseolus vulgaris*) and *Elsholtzia spendens* (Küpper *et al.* 2002, Pätsikkä *et al.* 2002, Peng *et al.* 2013). It is well known that salt stress is an important environmental factor that restricts plant growth and productivity (Boyer 1982). The decline in growth observed in many plants subjected to salt stress is often associated with a decrease in their photosynthetic capacity (Long and Baker 1986, Munns and Termaat 1986). Since it has been considered that one of the primary sites of damage to the photosynthetic apparatus by environmental stress is located in PSII (Baker 1991), the effects of salt stress on PSII in plants have been often investigated. Some studies have shown that salt stress inhibits PSII activity (Bongi and Loreto 1989, Belkhodja *et al.* 1994, Everard *et al.* 1994). In case of salt stress, our results were consistent with the report of Liu and Shen (2004) in spinach (*Spinacia oleracea*), where LHCII phosphorylation was inhibited, whereas it was enhanced in green alga (*Dunaliella salina*). Interestingly, in our study, the *PgCAB1* and *PgCAB2* expression levels significantly increased compared with control. This suggests the specific function of PSII in the regulatory role in ginseng response to salinity and drought which also might represent a strategy to prevent light stress-induced damage (Lu *et al.* 2002, 2003, El Rabey *et al.* 2015). The relative expression pattern of the five *PgCAB*s was inhibited under chilling stress (Fig. 5*D*). Terashima *et al.* (1994) reported that low temperature inhibits the activity of PSI much more than that of PSII in cucumber (*Cucumis sativus* L.), which correlates with our results. The *PgCAB*s associated to PSI (*PgCAB3*, *PgCAB4*, *PgCAB5*) showed lower expression compared to one *PgCAB* that belongs to PSII (*PgCAB1*). Capel *et al.* (1998) reported that low temperature regulates the expression of Lhcb in *Arabidopsis,* which suggests that our result can be useful in determining signal intermediates for a response to low temperature. The UV spectrum of solar radiation is known to modulate the structure and the primary photochemical reactions of thylakoid membranes (Joshi *et al.* 2013). UV radiation has adverse effects on chloroplasts (Greenberg *et al.* 1989, Jordan 1996, 2002; Bröovacs and Keresztes 2002, Kovacs and Keresztes 2002, Brösche and Strid 2003, Lidon *et al.* 2012). In case of UV radiation stress, the downregulation of *PgCAB1*, *PgCAB2*, *PgCAB4*, and *PgCAB5* (Fig. 5*D*) was consistent with the report that the photosynthetic activity in leaves treated with UV was significantly reduced in sweet potato (*Ipomoea batatas*) (Kim *et al.* 2007). The particular upregulation of *PgCAB3* suggests a specific function of each member of the CAB family in the regulation of the photosynthetic machinery in ginseng, which was consistent with other reports (Andersson *et al.* 2003, Xu *et al.* 2012). Interestingly, the intensity of the relative expression of *PgCAB1* and *PgCAB2* was very similar; as mentioned before, they belong to PSII, implying that PSII is downregulated. The response of *PgCAB*s to abiotic stresses might be related to the modulation of ROS homeostasis during photosynthesis (Xu *et al.* 2012).

In conclusion, five full-length cDNA sequences of ginseng *CAB* genes were identified and their expression patterns under different abiotic stresses were analyzed. Phylogenetic comparison of CAB members of ginseng, *Arabidopsis*, and higher plants revealed the putative functions of some ginseng CAB proteins. The present study provided useful information for further characterization of CABs.

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