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 photosystem (PS) I and II, 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 nicotinamide adenine dinucleotide phosphate ($NADP^+$) (Foyer and Noctor 1999). Light harvesting is the first step in the photosynthesis process, therefore the light-harvesting antenna has to be regulated in response to their physiological status and the environmental signals. Chlorophyll 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; 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 chlorophyll *a*-binding (CAA) proteins, the chlorophyll *a/b*-binding (CAB) proteins, the chlorophyll a/c-binding (CAC) proteins, and the lesser known LHC clades including LHCx and LHCz. Higher

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Abbreviations: CAB – chlorophyll *a/b*-binding protein; 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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plants possesses only CAB, therefore CAB is regarded as LHC, light-harvesting chlorophyll 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 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 chlorophyll in the plants, indicating its importance in plants. It is particularly interesting because of its involvement in both short- and long-term adaptation to different light and temperature conditions.

Since the identification of Lhc gene in pea (*Pisum sativum*) (Badbrook *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 through Lhca4) and six with PSII (Lhcb1 through 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 through Lhca4), and ten with PSII (Lhcb1 through Lhcb6) (Hoffman *et al.* 1987, Pichersky *et al.* 1985, 1987, 1988, 1989, 1991, 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 it is cultivated for its highly valued root used for medicinal purposes. Previously, we reported one CAB gene (In *et al.* 2005), but no other report on CAB gene in ginseng exists, despite the importance of the light sensitivity of ginseng (Harding 1908, Parmenter *et al.* 2000, In *et al.* 2010). It needs to be cultivated under special conditions to meet its requirements of about 30% full sunlight (Kim *et al.* 2014a) growing 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 to contribute to the understanding of the possible role of ginseng CAB.

Materials and methods

Nucleotide sequencing and sequence analysis: To identify genes from the previously constructed expressed sequence tags (EST) libraries from 4-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 CABs 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 cultured plantlets were used for the 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 the indicated concentrations of chemicals: 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 UVC 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 16 h of light and 8 h of dark. The stressed plant materials from all completed treatments were immediately frozen in liquid nitrogen and stored at −70°C until required.

For the light and dark treatment, three-year-old ginseng plants hydroponically grown in a controlled greenhouse were used for dark treatment. Control plants were grown in 16 h of light and 8 h of dark and sampled in light conditions, whereas dark treatments (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 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 closed to zero, indicating that the efficiencies of the gene and the internal control β-actin were equal.

Statistical analysis: Means of three independent replicates are 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* chlorophyll *a/b*-binding protein). The corresponding characteristics of each *PgCAB*s are indicated in Table 1. The full-length cDNA sequences have been assigned to GenBank under the accession numbers (KP874095-KP874099). In addition, the tertiary structure of matured proteins without transit peptides are 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 most conserved within the PgCABs was also identified in each model that contains the conserved LHC motif.

Table 1. Characteristics of ginseng CABs. [a] 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[a] [amino acids]	Molecular mass[a] [kDa]	pI[a]
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).

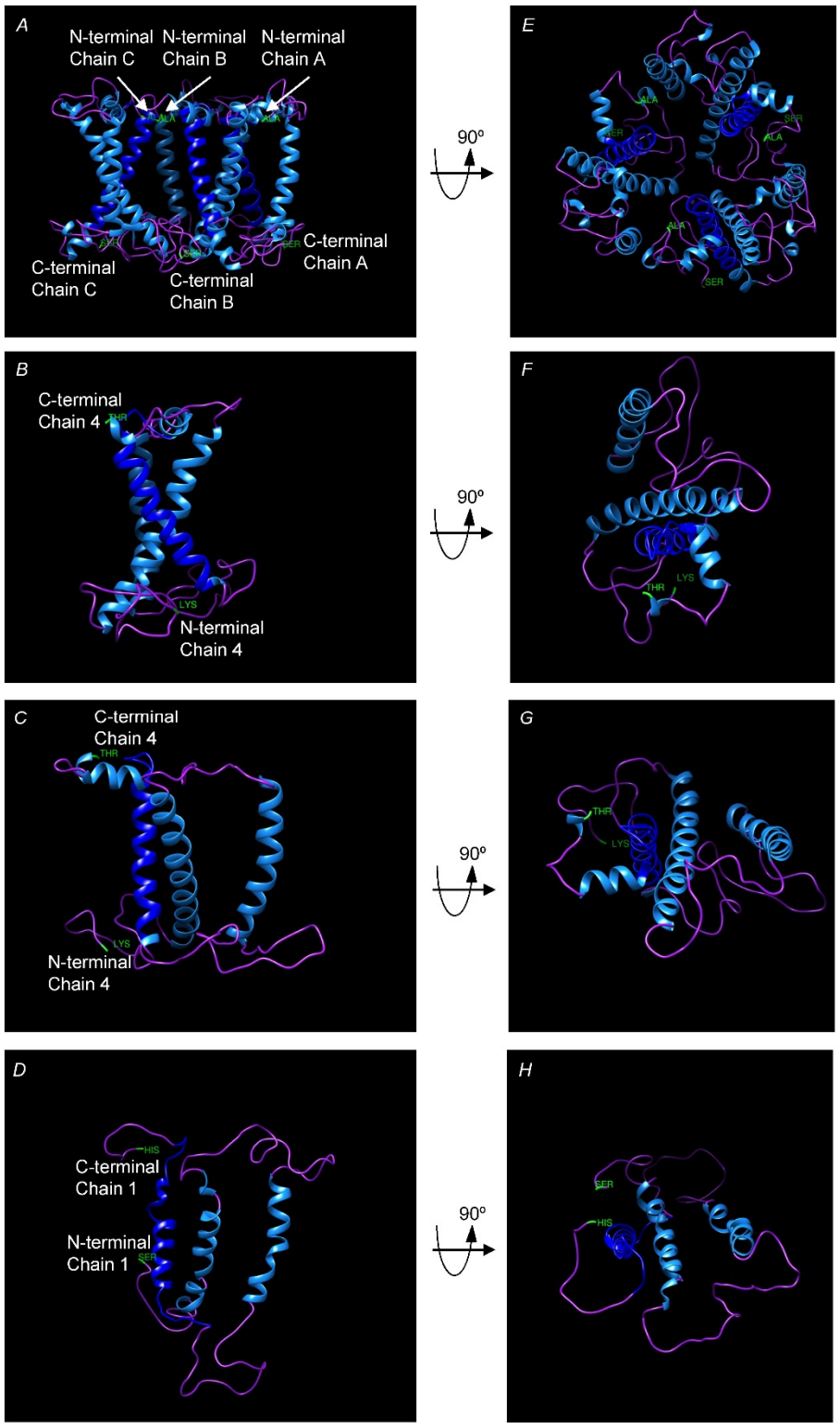

Fig. 1. The predicted 3-D structures of PgCABs. Comparative representation was performed by UCSF Chimera package and helix and coil structures are depicted as sky-blue and purple, respectively. Motif 2 protein sequences analyzed by MEME are depicted as blue. The top view of *A*: PgCAB1, *B*: PgCAB3,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

Since the complete set of CAB genes has been characterized from *Arabidopsis*, although many reports of one type of CAB gene (mostly LHCII family) from various species have sequenced, 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*

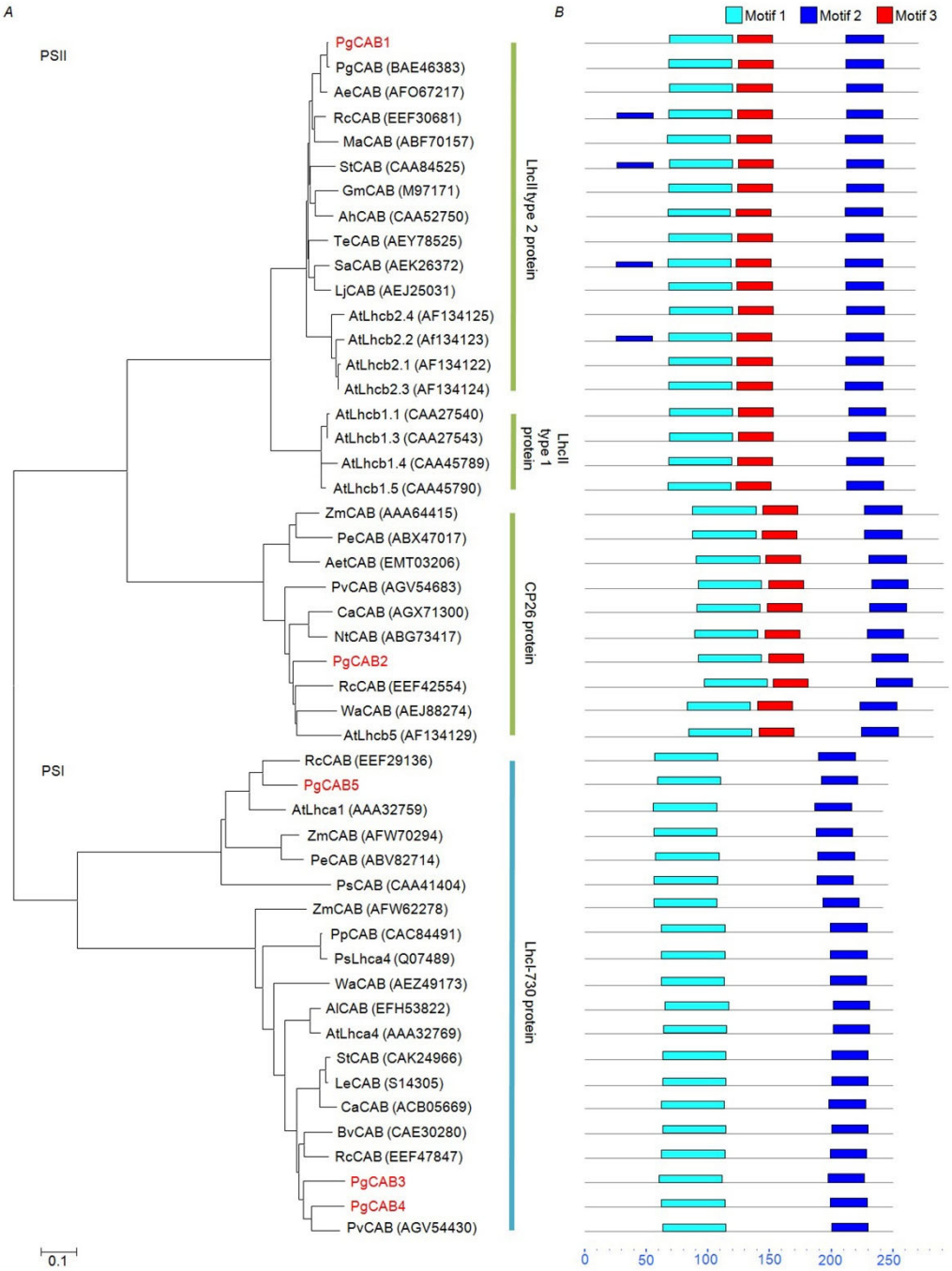

Fig. 2. *A*: Sequence homology analysis of *PgCABs* with LHC proteins associated with Arabidopsis and higher plants. A phylogenetic tree of *PgCAB1*, *PgCAB2*, *PgCAB3*, *PgCAB4*, and *PgCAB5* (in bold red letter). The neighbor-joining method was used, and the branch lengths are proportional to the divergence, 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 Supplements Figure 2S. 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.

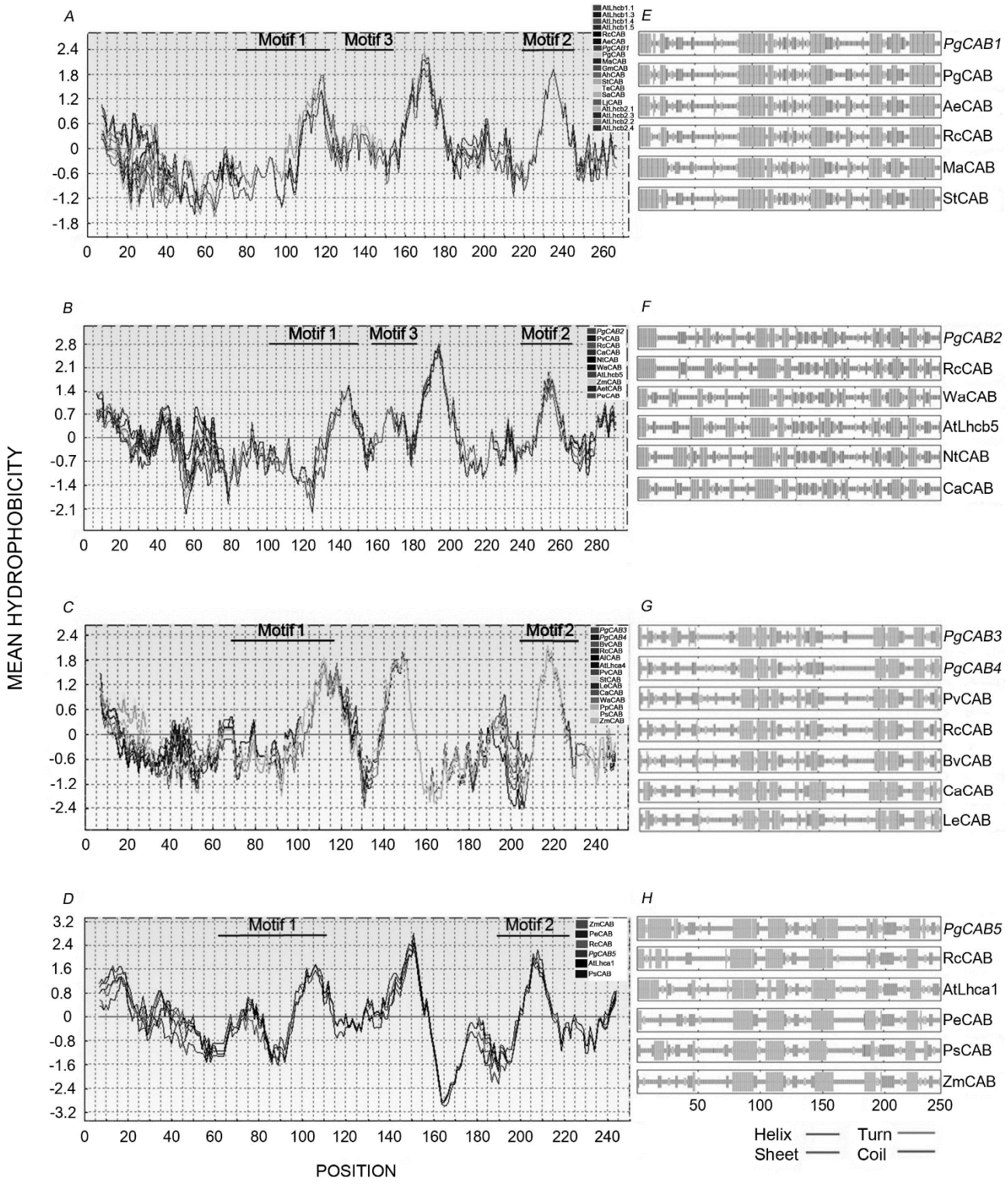

Fig. 3. Superimposed hydrophobicity profiles and secondary structure predictions for each PgCAB group and homologous. Hydrophobic domains are indicated by positive numbers, hydrophilic domains are above the line and hydrophilic domains are below the line. *A*: PgCAB1 group associated with AtLhcb2, *B*: 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.

genes. In addition, conserved motifs were found by MEME analysis in all plant CAB isozymes (Fig. 2*B*). There are two motifs in the PgCABs associated to 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 'ELINGRLAMLGFLGFLVPELIT' (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 is 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).

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.

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

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

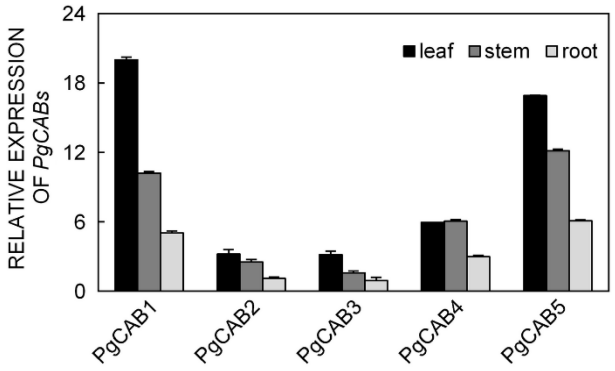

Fig. 4. Expression of *PgCAB* genes in leaves, stem, and roots of 3 years old *Panax ginseng.* Bars indicate the mean value ± SE from three independent experiments.

Temporal expression of *PgCAB* genes in response to abiotic stresses: The expression patterns of *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, dark condition caused significant decrease of *PgCABs* (Fig. 5*A*).

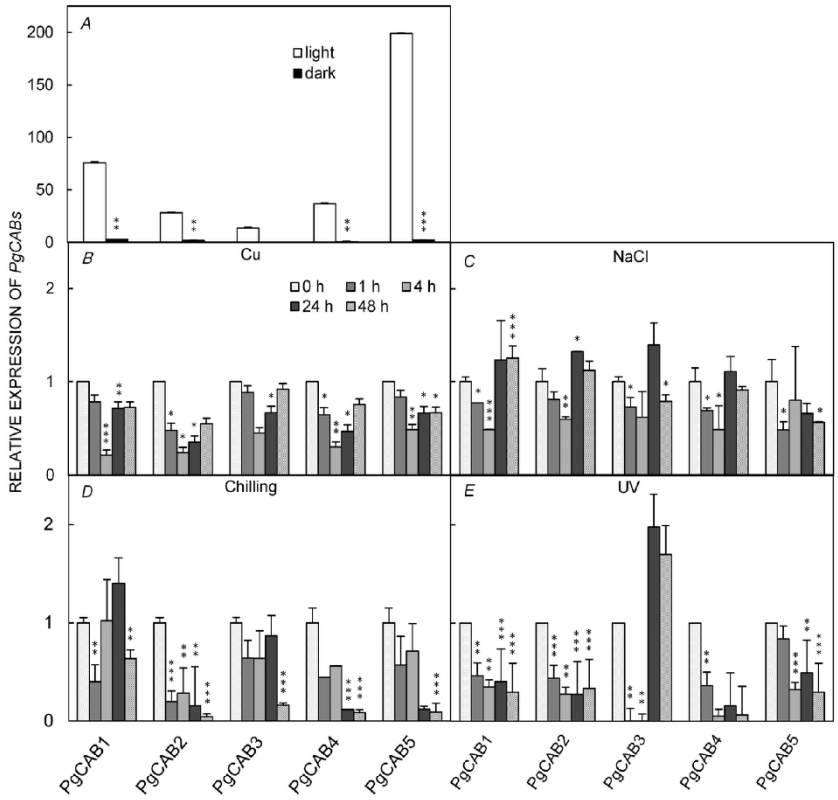

Fig. 5. Relative quantities of *PgCAB1, PgCAB2, PgCAB3, PgCAB4,* and *PgCAB5* mRNA at various time points post-treatments with various stresses: *A*: light-dark, *B*: 100 mM $CuSO_4$, *C*: 100 mM NaCl, *D*: chilling, *E*: UV light. Bars indicate the mean value ± SE from three independent experiments.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

Following the copper stress (Fig. 5*B*), *PgCABs* expression level was reduced compared to control. Following the NaCl stress (Fig. 5*C*), *PgCAB1* was extremely up-regulated twice at 24 and 48 h, while *PgCAB2*, was significantly up-regulated twice at 24 and 48 h after treatment, whereas *PgCAB3*, and *PgCAB4* significantly increased at 24 h and decreased at 48 h after treatment, while *PgCAB5* was significantly decreased compared to control. Following the chilling stress (Fig. 5*D*), *PgCAB1* gene expression increased to highest expression level at 24 h and decreased very significantly at 48 h after treatment, while *PgCAB3* gene expression increased to highest expression level at 24 h and decreased very significantly at 48 h after treatment, whereas *PgCAB2*, *PgCAB4*, and *PgCAB5* gene expression was 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 treatment, whereas the other *PgCABs* gene expression was very significantly declined at 48 h after treatment compared to control.

Discussion

The sunlight is the primary energy source of our planet, and the photosynthetic machineries have been investigated in great details from plants, algae, and photosynthetic bacteria. 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, because it requires low irradiance of light. High light can damage ginseng leaves easily (Kim *et al.* 2006). Therefore, we aim to investigate the light-harvesting CAB genes regarding with light and abiotic stresses. Interestingly, Kim *et al.* (2006) reported much higher abundance of CAB in ginseng leaf cDNA library compared with other plants, such as Stevia and rice ESTs, indicating important and unique expression pattern of CABs in ginseng leaf. However, only one PgCAB was reported previously (In *et al.* 2006), and further characterization and grouping has not been performed in *P. ginseng*.

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 associated with PSI are named Lhca, and the ones associated to PSII are named Lhcb (Engelken *et al.*

2012), they 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). 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, which 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 will 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*. 2004, Ruban *et al*. 2007). Lhcb5, also called CP26, is probably monomeric protein that is present in one copy per PSII unit (Jansson 1999). The possess 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 and is known as less important catalytic activity function (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 in dry seeds (Matsuoka 1990, Xu *et al*. 2012), and the high expression of *PgCAB*s in leaves is consistent with the localization of the photosynthetic machineries. 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 the expression levels; LHCII type1, particularly Lhcb1.3 is expressed at the highest level, whereas LHCII type 2 family and Lhcb4.3, Lhca5-6 are expressed at a low level (Janssen 1999). The higher expression level of *PgCAB1* compared with other *PgCAB*s in ginseng leaves is different with 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 leaf, there is evidence reported that CAB genes are expressed 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 of sugar beet (*Beta vulgaris* L.) in leaves and roots; and Basset *et al.* (2007) reported the expression of CAB of peach (*Prunus persica* L.) in leaves and roots. Our results confirmed that the five *PgCAB*s were also expressed in stem and root (Fig. 4). However, *PgCAB* genes are expressed not only in green tissues, being appropriate to discuss how distinct regulation patterns might reflect the expression of *PgCABs* in tissues other than green. There are two possible explanations for this finding: that the tissues sampled included a few chloroplast-containing cells or that some Lhc genes are also expressed at very low levels in cells lacking chloroplasts (Klimmek *et al.* 2006).

The highest expression of *PgCAB5* in three-year-old mature leaf (Fig. 4), showing difference with expression pattern in 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 to regulate *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 2010). Corresponding with dark-dependent inhibition of *CAB* expression (Matsuoka 1990), the five *PgCAB*s showed very low expression level in dark condition (Fig. 5*A*), confirming the light-dependent expression of all *PgCAB* genes in leaf.

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 play 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 like salinity (Munns and Termaat 1986, Munns 2005), metal ion variation (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 to control; under NaCl stress *PgCAB1*, *PgCAB2*, *PgCAB3*, and *PgCAB4* were enhanced at 24 h after treatment, except *PgCAB1* and *PgCAB2*, both belonging to PSII, were up-regulated at 48 h compared control; under chilling stress only *PgCAB1* was enhanced at 24 h after treatment compared to control; and under UV stress only *PgCAB3* was up-regulated compared to 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 pattern 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 (*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 investigated by many studies. 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 data is 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 *PgCAB1* and *PgCAB2* expression level was significantly increased compared to control, suggesting 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*C*); Terashima *et al.* (1994) reported that low temperature inhibit 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*), although Capel *et al*. (1998) reported that low temperature regulates the expression of Lhcb in Arabidopsis, suggesting that our result can be useful in determining signal intermediates in low temperature response. 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*) is consistent with the report that the photosynthetic activity in leaves treated with UV was significantly reduced in sweetpotato (*Ipomoea batatas*) (Kim *et al.* 2007) the particular upregulated response of *PgCAB3* suggests the specific function of each member of the CAB family in the regulation of the photosynthetic machinery in ginseng, which is consistent with other reports (Andersson *et al.* 2003, Xu *et al.* 2011). Interestingly, the intensity of the relative expression of *PgCAB1* and *PgCAB2* is very similar, as mentioned before they belong to PSII, implying that PSII is down-regulated. The response of *PgCAB*s to abiotic stresses is probably modulation of ROS homeostasis during photosynthesis (Xu *et al.* 2011).

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

References

Allen J.F., De Paula W.B.M., Puthiyaveetil S., Nield J.: A structural map for chloroplast photosynthesis. – Trends Plant Sci, **16**: 645-655, 2011.

Andersson J., Wentworth M., Walters R.G. *et al*.: Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II – effects on photosynthesis, grana stacking and fitness. – Plant J. **35**: 350-361, 2003.

Arnold J., Bordoli L., Kopp J., Schwede T.: The SWISS-MODEL Workspace. A web-based environment for protein structure homology modeling. – Bioinformatics **22**: 195-201, 2006.

Bailey T.L., Boden M., Buske F.A. *et al*.: MEME SUITE: tools for motif discovery and searching. – Nucl. Acids. Res. **37**: 202-208, 2009.

Baker N.R.: A possible role for photosystem II in environmental perturbations of photosynthesis. – Physiol. Plant. **81**: 563-570, 1991.

Bannai H., Tamada Y., Maruyama O. *et al.*: Extensive feature detection of N-terminal protein sorting signals. – Bioinformatics. **18**: 298-305, 2002.

Bassett C.L., Callahan A.M.: Characterization of a type II chlorophyll a/b-binding protein gene (Lhcb2*Pp1) in peach. II. mRNA abundance in developing leaves exposed to sun or shade. – Tree Physiol. **23**: 473-480, 2003.

Bassi R., Sandonà D., Croce R.: Novel aspects of chlorophyll a/b-binding proteins. – Physiol. Plant. **100**: 769-779, 1997.

Belkhodja R., Morales F., Abadia A. *et al*.: Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). – Plant Physiol. **104**: 667-673, 1994.

Bergantino E., Dainese P., Cerovic Z. *et al*.: A post-translational modification of the photosystem II subunit CP29 protects maize from cold stress. – J. Biol. Chem. **270**: 8474-8481, 1995

Bongi G., Loreto F.: Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. - Plant Physiol. **90**: 1408-1416, 1989.

Boyer J.S.: Plant productivity and environment. – Science **218**: 443-448, 1982.

Brösche M., Strid A.: Molecular events following perception of ultraviolet-B radiation by plants. - Physiol. Plant. **117**: 1-10, 2003.

Capel J., Jarillo J.A., Madueño F. *et al*.: Low temperature regulates Arabidopsis Lhcb gene expression in a light-independent manner. – Plant J. **13**: 411-418, 1998.

Chitnis P.R.: Photosystem I: function and physiology. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **52**: 593-626, 2001.

De Montaigu A., Toth R., Coupland G.: Plant development goes like clockwork. – Trends Genet. **26**: 296-308, 2010.

Gobets B., van Grondelle R.: Energy transfer and trapping in photosystem I. – Biochim. Biophys. Acta Bioenerg. **1507**: 80-99, 2001.

El Rabey H.A., Al-Malki A.L., Abulnaja K.O., Rhode W.: Proteome analysis for understanding abiotic stress (salinity and drought) tolerance in Date Palm (*Phoenix dactylifera* L.). – Int. J. Genomics. **2015**: 1-11, 2015.

Engelken J., Brinkmann H., Adamska I.: Taxonomic distribution and origins of the extended LHC (light-harvesting complex) antenna protein superfamily. – BMC Evol. Biol. **10**, 2010.

Engelken J., Funk C., Adamska I.: The extended light-harvesting complex (Lhc) protein superfamily: Classification and evolutionary dynamics.-R.L. Burnap and W.F.J. Vermaas (eds.), Functional Genomics and Evolution of Photosynthetic Systems, 265 – Adv. Photosynth. Respir. **33**: 265-284, 2012.

Everard J.D., Gucci R., Kann S.C. *et al*.: Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. – Plant Physiol. **106**: 281-292, 1994.

Fischer N., Boudreau E., Hippler M. *et al*.: A large fraction of PsaF is nonfunctional in photosystem I complexes lacking the PsaJ subunit. – Biochem. **38**: 5546-5552, 1999.

Foyer C.H., Noctor G.: Leaves in the dark see the light. – Science. **284**: 599-601, 1999.

Galvez-Valdivieso G., Mullineaux P.M.: The role of reactive oxygen species in signaling from chloroplasts to the nucleus. – Physiol. Plant. **138**: 430-439, 2010.

Gasteiger E., Hoogland C., Gattiker A. *et al*.: Protein identification and analysis tools on the ExPASy server. – In: Walker, John M. (Ed.), The Proteomics Protocols Handbook. Humana Press, pp. 571-607, 2005.

Geourjon C., Deleage G.: SOPMA: significant improvements in protein secondary structure prediction by consensus prediction form multiple alignments. – Comput. Appl. Biosci. **11:**681-684, 1995.

Green B.R., Durnford F.G.: The chlorophyll-carotenoid proteins of oxygenic photosynthesis. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **47**: 685-714, 1996.

Green B.R., Pichersky E., Kloppstech K.: Chlorophyll a/b-binding proteins: an extended family. – Trends Biochem. Sci. **16**: 181-186, 1991.

Greenberg B.M., Gaba V., Canaani O. *et al*.: Separate photosensitizers mediate degradation of the 32 kDa photosystem II reaction centre protein in the visible and UV spectral regions. – Proc. Natl. Acad. Sci. USA. **86**: 2865-2869, 1989.

Harding A.R.: Ginseng and other medicinal plants. Published by A. R. Harding. Re-issued with additions by Emporium Publications, Boston, Mass. 1972. 367 p., 1908.

Henrysson T., Schröder W.P., Spangfort M., Akerlund H.E.: Isolation and characterization of the chlorophyll a/b protein complex CP29 from spinach. – Biochim. Biophys. Acta. **977**: 301-308, 1989.

Hoffman N.E., Pichersky E., Malik V.S. *et al*.: A cDNA clone encoding a photosystem I protein with homology to photosystem II chlorophyll a/b-binding polypeptides. – Proc. Natl. Acad. Sci. USA. **84**: 8844-8848, 1987.

Horton P., Ruban A.: Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. – J. Exp. Bot. **56**: 365-373, 2004.

Humbeck K., Krupinska K.: The abundance of minor chlorophyll a/b-binding proteins CP29 and LHCI of barley (*Hordeum vulgare* L.) during leaf senescence is controlled by light. – J. Exp. Bot. **54**: 375-383, 2003.

In J.G., Lee B.S., Youn J.H. *et al*.: Molecular characterization of a cDNA encoding chlorophyll a/b binding protein (cab) form *Panax ginseng* C. A. Meyer. – Korean J. Plant Res. **18**: 441-449, 2005.

In J.G., Kim M.K., Lee O.R. *et al*.: Molecular identification of Korean mountain ginseng using an Amplification Refractory Mutation System (ARMS). – J. Ginseng Res. **34**: 41-46, 2010.

Jansson S., Pichersky E., Bassi R. *et al*.: A nomenclature for the genes encoding the chlorophyll a/b-binding proteins of higher plants. – Plant Mol. Biol. Rep. **10**: 242-253, 1992.

Jansson S.: A guide to the identification of the Lhc genes and their relatives in *Arabidopsis*. – Trends Plant. Sci., **4**: 236-240, 1999.

Jansson S.: A protein family saga: From photoprotection to light-harvesting (and back?). – In: Demmig-Adams B, Adams W.W.III., and Mattoo A.K. (eds) Photoprotection, photoinhibition, gene regulation and environment, pp. 145-153. Springer, Dordrecht, 2006.

Jansson S., Green B., Grossman A.R., Hiller R.: A proposal for extending the nomenclature of light-harvesting proteins of the three transmembrane helix type. – Plant Mol. Biol. Report. **17**: 221-224, 1999.

Jensen P.E., Bassi R., Boekema E.J. *et al*.: Structure, function and regulation of plant photosystem I. – Biochim. Biophys. **1767**: 335-352, 2007.

Jordan B.R.: The effects of UV-B radiation on plants: a molecular perspective. – Adv. Bot. Res. **22**: 97-162, 1996.

Jordan B.R.: Molecular response of plant cells to UV-B stress. – Funct. Plant Biol. **29**: 909-918, 2002.

Joshi P.N., Biswal B., Kulandaivelu G., Biswal U.C.: Response of senescing wheat leaves to ultraviolet-A light: changes in energy transfer efficiency and PSII photochemistry. – Radiat. Environ. Biophys. **33**: 167-176, 1994.

Joshi P.N., Gartia S., Pradhan M.K., Biswal B.: Photosynthetic response of clusterbean chloroplasts to UV-B radiation: energy imbalance and loss in redox homeostasis between Q_A and Q_B of photosystem II. – Plant. Sci. **181**: 90-95, 2011.

Joshi P.N., Misra A.N., Nayak L., Biswal B.: Response of mature, developing and senescing chloroplast to environmental stress. – In: Plastid development in leaves during growth and senescence: B. Biswal, Krupinska, K., Biswal, U. C., (Eds.). – Adv. Photosynth. Respir. **36**: 641-668, 2013.

Joshi P.N., Ramaswamy N.K., Raval M.K. *et al*.: Response of senescing leaves of wheat seedlings to UV-A radiation: inhibition of PSII activity in light and darkness. – Environ. Exp. Bot. **38**: 237-242, 1997.

Kim C., Choo G.C., Cho H.S., Lim J.T.: Soil properties of cultivation sites for mountain-cultivated ginseng at local level. – J. Ginseng Res. **39**: 76-80, 2015.

Kim M.K., Lee B.S., In, J.G. *et al*.: Comparative analysis of expressed sequence tags (ESTs) of ginseng leaf. – Plant Cell Rep. **25**: 599-606, 2006.

Kim Y.H., Lim S., Han S.H. *et al*.: Differential expression of 10 sweetpotato peroxidades in response to sulfur dioxide, ozone, and ultraviolet radiation. – Plant Physiol. Biochem. **45**: 908-914, 2007.

Kim Y.J., Jeon J.N., Jang M.G. *et al*.: Ginsenoside profiles and related gene expression during foliation in *Panax ginseng* Meyer. – J. Ginseng Res. **38**: 66-72, 2014a.

Kim Y.J., Lee O.R., Oh J.Y. *et al*.: Functional analysis of HMGRs in biosynthesizing triterpene saponin in *Panax ginseng*. – Plant Physiol. **165**: 1-16, 2014b.

Kim Y.J., Shim J.S., Krishna P.R. *et al*.: 2008. Isolation and characterization of a glutaredoxin gene from *Panax ginseng* C A. Meyer. – Plant Mol. Biol. Report. 26, 335-349.

Klimmek F., Sjödin A., Noutsos C. *et al*.: Abundantly and rarely expressed Lhc protein genes exhibit distinct regulation patterns in plants. – Plant Physiol. **140**: 793-804, 2006.

Kovacs E., Keresztes A.: Effect of gamma and UV-B/C radiation on plant cells. – Micron. **33**: 199-210, 2002.

Krasensky J., Jonak C.: Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. – J. Exp. Bot. **63**: 1593-1608, 2012.

Küpper H., Setlík I, Spiller M., *et al*.: Heavy metal-induced inhibition of photosynthesis: targets of in vivo metal chlorophyll formation. – J. Phycol. **38**: 429-441, 2002.

Kuttkat A. Hartmann A., Hobe S., Paulsen H.: The C-terminal domain of light-harvesting chlorophyll a/b-binding protein is involved in the stabilization of trimeric light-harvesting complex. – Eur. J. Biochem. **242**: 288-292, 1996.

Kwak J.M., Nguyen V., Schroeder J.I.: The role of reactive oxygen species in hormonal responses. – Plant Physiol. **141**: 323-329, 2006.

Kyte J., Doolittle R.F.: A simple method for displaying the hydropathic character of a protein. – J. Mol. Biol. **157**: 105-132, 1982.

Larbi A., Abadia A., Abadia J., Morales F.: Down co-regulation of light absorption, photochemistry, and carboxylation in Fe-deficient plants growing in different environments. – Photosynth. Res. **89**: 113-126, 2006.

Lidon F.J.C., Reboredo F.H., Leitão A.E. *et al*.: Impact of UV- B radiation on photosynthesis-an overview. – Emir. J. Food Agric. **24**: 546-556, 2012.

Liu X.D., Shen Y.G.: NaCl-induced phosphorylation of light harvesting chlorophyll a/b proteins in thylakoid membranes from the halotolerant green alga, *Dunaliella salina.* – FEBS Lett. **569**: 337-340, 2004.

Livak K.J., Schmittgen T.D.: Analysis of relative gene expression data using realtime quantitative PCR and the 2(-delta delta C(T)) methods. – Methods. **25**: 402-408, 2001.

Lu C., Jiang G., Wang B., Kuang T.: Photosystem II photochemistry and photosynthetic pigment composition in salt-adapted halophyte *Artimisia anethifolia* grown under outdoor conditions. – J. Plant Physiol. **160**: 403-408, 2003.

Lu C., Qiu N., Lu Q. *et al*.: Does salt stress lead to increased susceptibility of photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte *Suaeda salsa* grown outdoors?. – Plant Sci. **163**: 1063-1068, 2002.

Matsuoka M.: Classification and characterization of cDNA that encodes the light harvesting chlorophyll a/b-binding protein of photosystem II from Rice. – Plant Cell Physiol. **31**: 519-526, 1990.

Melkozernov A.N.: Excitation energy transfer in photosystem I from oxygenic organisms. – Photosynth. Res. **70**: 129-153, 2001.

Millar A.J., Kay S.: Integration of circadian and phototransduction pathways in the network controlling CAB gene transcription in Arabidopsis. – Proc. Natl. Acad. Sci. USA. **93**: 15491-15494, 1996.

Munns R.: Genes and salt tolerance: bringing them together. – New. Phytol. **167**: 645-663, 2005.

Munns R., Termaat A.: Whole plant responses to salinity. – Aust. J. Plant Physiol. **13**: 143-160, 1986.

Nield J., Redding K., Hippler M.: Remodeling of light-harvesting protein complexes in Chlamydomonas in response to environmental changes. – Eucaryotic Cell. **3**: 1370-1380, 2004.

Nott A., Jung H.S., Koussevitzky S., Chory J.: Plastid-to-nucleus retrograde signaling. – Annu. Rev. Plant Biol. **57**: 739-759, 2006.

Palomares R., Herrmann R.G., Oelmüler R.: Different blue-light requirement for the accumulation of transcripts from nuclear genes for thylakoid proteins in *Nicotiana tabacum* and *Lycopersicon esculentum*. – J. Photochem. Photobiol. B. **11**: 151-162, 1991.

Parmenter G., Littlejohn R.: Effect on shade on growth and photosynthesis of *Panax ginseng.* - New Zeal. J. Crop Hort. **28**: 255-269, 2000.

Pätsikkä E., Kairavuo M., Sersen F. *et al*.: Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. – Plant Physiol. **129**: 1359-1367, 2002.

Peer W., Silverthorne J., Peters J.: Developmental and light-regulated expression of individual members of the light-harvesting complex b gene family in *Pinus palustris*. – Plant Physiol. **111**: 627-634, 1996.

Peng H., Kroneck P.M., Küpper H.: Toxicity and deficiency of copper in *Elsholtzia splendens* affect photosynthesis biophysics, pigments and metal accumulation. – Environ. Sci. Technol. **47**: 6120-6128, 2013.

Pichersky E., Brock T.G., Nguyen D. *et al*.: A new member of the CAB gene family: structure, expression and chromosomal location of Cab-8, the tomato gene encoding the Type III chlorophyll a/b-binding polypeptide of photosystem I. – Plant Mol. Biol. **12**: 257-270, 1989.

Pichersky E., Bernatzky R., Tanksley S.D. *et al*.: Molecular characterization and genetic mapping of two clusters of genes encoding chlorophyll a/b-binding proteins in *Lycopersicon esculentum* (tomato). – Gene **40**: 247-258, 1985.

Pichersky E., Hoffman N.E., Malik V.S. *et al*.: The tomato Cab-4 and Cab-5 genes encode a second type of CAB polypeptides localized in photosystem II. – Plant Mol. Biol. **9**: 109-120, 1987.

Pichersky E., Soltis D., Soltis P.: Defective chlorophyll a/b-binding protein genes in the genome of a homosporous fern. – Proc. Natl. Acad. Sci. U.S.A. **87**: 195-199, 1990.

Pichersky E., Subramaniam R., White M.J. *et al*.: Chlorophyll a/b binding (CAB) polypeptides of CP29, the internal chlorophyll a/b complex of PSII: characterization of the tomato gene encoding the 26 kDa (type I) polypeptide, and evidence for a second CP29 polypeptide. – Mol. Gen. Genet. **227**: 277-284, 1991.

Pichersky E., Tanksley S.D., Piechulla B. *et al*.: Nucleotide sequence and chromosomal location of Cab-7, the tomato gene encoding the type II chlorophyll a/b-binding polypeptide of photosystem I. – Plant Mol. Biol. **11**: 69-71, 1988.

Pruneda-Paz J.L., Kay S.A.: An expanding universe of circadian networks in high plants. – Trends Plant Sci. **15**: 259-265, 2010.

Ruban A.V., Berera R., Ilioaia C. *et al*.: Identification of a mechanism of photoprotective energy dissipation in higher plants. – Nature. **450**: 575-578, 2007.

Schwartz E., Pichersky E.: Sequence of two tomato nuclear genes encoding chlorophyll a/b-binding proteins of CP24, a PSII antenna component. – Plant Mol. Biol. **15**: 157-160, 1990.

Schwartz E., Shen D., Aebersold R. *et al*.: Nucleotide sequence and chromosomal location of Cab11 and Cab12, the genes for the fourth polypeptide of the photosystem I light-harvesting antenna (LHCI). – FEBS Lett. **280**: 229-234, 1991.

Schwartz E., Stasys R., Aebersold R. *et al*.: Sequence of a tomato gene encoding a third type of LHCII chlorophyll a/b-binding polypeptide. – Plant Mol. Biol. **17**: 923-925, 1991.

Silverthorne J., Tobin E.M.: Demonstration of transcriptional regulation of specific genes by phytochrome action. – Proc. Natl. Acad. Sci. USA. **81**: 1112-1116, 1984.

Spangfort M., Larsson U.K., Ljungberg U. *et al*.: The 20 kDa apo-polypeptide of the chlorophyll a/b protein complex CP24 - Characterization and complete primary amino sequence. – In: Current Research in Photosynthesis. Vol. 2. Baltscheffsky M (ed) Kluwer Academic Publishers, pp. 253-256, 1990.

Stahl D.J, Kloos D.U., Hehl R.: A sugar beet chlorophyll a/b binding protein promoter void of G-box like elements confers strong and leaf specific reporter gene expression in transgenic sugar beet. – BMC Biotech. **4**, 2004.

Staneloni R.T., Rodriguez-Batiller M.J., Casal J.J.: Abscisic acid, high-light, and oxidative stress down-regulate a photosynthetic gene via a promoter motif not involved in phytochrome-mediated transcriptional regulation. – Mol. Plant. **1**: 75-83, 2008.

Sun L., Tobin E.M.: Phytochrome-regulated expression of genes encoding light-harvesting chlorophyll a/b-binding protein in two long hypocotyls mutants and wild type plants of *Arabidopsis thaliana*. – Photochem. Photobiol. **52**: 51-56, 1990.

Tao L., Zeba N., Ashrafuzzaman M., Hong C.B.: Heavy metal stress-inducible early light-inducible gene CaELIP from hot pepper *(Capsicum annuum)* shows broad expression patterns under various abiotic stresses and circadian rhythmicity. – Environ. Exp. Bot. **72**: 297-303, 2011.

Terashima I., Funayama S., Sonoike K.: The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperatures is photosystem I, not photosystem II. – Planta **193**: 300-306, 1994.

Thines B., Harmon F.G.: Four easy pieces: mechanisms underlying circadian regulation of growth and development. – Curr. Opin. Plant Biol. **14**: 1-7, 2010.

Vass I., Turcsanyi E., Touloupakis E. *et al*.: The mechanism of UV-A radiation induced inhibition of photosystem II electron transport studied by EPR and Chl fluorescence. – Biochemistry. **41**: 10200-10208, 2002.

Weatherwax S.C., Ong M.S., Degenhardt J. *et al*.: The interaction of light and abscisic acid in the regulation of plant gene expression. – Plant Physiol. **111**: 363-370, 1996.

Wientjes E., Amerongen H.V., Croce R: LHCII is an antenna of both photosystems after long-term acclimation. – Biochim. Biophys. Acta. **1827**: 420-426, 2013.

Wollman F.A.: State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. – EMBO J. **20**: 3623-3630, 2001.

Xu Y.H., Liu R., Yan L. *et al*.: Light-harvesting chlorophyll a/b-binding proteins are required for stomatal response to abscisic acid in *Arabidopsis*. – J. Exp. Bot. **63**: 1095-1106, 2012.

Yang D.H., Webster J., Adam Z. *et al*.: Induction of acclimative proteolysis of the light-harvesting chlorophyll a/b protein of photosystem II in response to elevated light intensities. – Plant Physiol. **118**: 827-834, 1998.