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Research article

cis-Prenyltransferase interacts with a Nogo-B receptor homolog for dolichol biosynthesis in *Panax ginseng* Meyer

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ABSTRACT

Background: Prenyltransferases catalyze the sequential addition of isopentenyl diphosphate units to allylic prenyl diphosphate acceptors and are classified as either *trans*-prenyltransferases (TPTs) or *cis*-prenyltransferases (CPTs). The functions of CPTs have been well characterized in bacteria, yeast, and mammals compared to plants. The characterization of CPTs also has been less studied than TPTs. In the present study, molecular cloning and functional characterization of a CPT from a medicinal plant, *Panax ginseng* Mayer were addressed.

Methods: Gene expression patterns of *PgCPT1* were analyzed by quantitative reverse transcription polymerase chain reaction. *In planta* transformation was generated by floral dipping using *Agrobacterium tumefaciens*. Yeast transformation was performed by lithium acetate and heat-shock for *rer2Δ* complementation and yeast-two-hybrid assay.

Results: The ginseng genome contains at least one family of three putative *CPT* genes. *PgCPT1* is expressed in all organs, but more predominantly in the leaves. Overexpression of *PgCPT1* did not show any plant growth defect, and its protein can complement yeast mutant *rer2Δ* via possible protein–protein interaction with PgCPTL2.

Conclusion: Partial complementation of the yeast dolichol biosynthesis mutant *rer2Δ* suggested that PgCPT1 is involved in dolichol biosynthesis. Direct protein interaction between PgCPT1 and a human Nogo-B receptor homolog suggests that PgCPT1 requires an accessory component for proper function.

1. Introduction

Isoprenoid biosynthesis generally occurs by condensation of the five-carbon (C5) isoprene building block, isopentenyl diphosphate (IPP), which is synthesized by two distinct pathways [1,2]. IPP is synthesized via the mevalonate (MVA) pathway in the cytosol and the 2-*C*-methyl-D-erythritol 4-phosphate pathway in the plastids [3]. Successive condensation of IPP with allylic prenyl diphosphates initiates isoprenoid synthesis and leads to the synthesis of a series of linear prenyl diphosphates called polyisoprenoids, which typically range in size from C15 to C120 [4]. The sequential addition of IPP units to allylic prenyl diphosphate acceptors is catalyzed by the action of enzymes called prenyltransferases. Depending on the stereochemistry of the polyisoprenoids, the prenyltransferases are classified as either *trans*-prenyltransferases (TPTs or [*E*]-prenyl diphosphate synthases) or *cis*-prenyltransferases [CPTs, also referred to as dehydrodolichyl diphosphate synthase, [*Z*]-prenyl diphosphate synthase, or undecaprenyl diphosphate synthase (UPPS)]. Although CPTs and TPTs share similar substrate preferences and reaction products, they are easily distinguished by their primary amino acid sequence [5,6].

Since 1987, genes encoding *trans*-prenyltransferases have been cloned and characterized [7]. They are involved in the synthesis of geranyl pyrophosphate (GPP, C10); *trans*, *trans*-farnesyl pyrophosphate, (C15); all-*trans*-geranylgeranyl pyrophosphate (C20); and solanesyl pyrophosphate (C45). However, studies on the biological function of CPTs are generally lacking. The CPTs are divided into three groups based on the specific final chain length: short-chain (3 isoprene units); medium-chain (10 or 11 isoprene units); and long-chain (14–24 isoprene units) [7,8]. The functions of CPTs have been

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identified in bacteria, yeast, and mammals [9–11]. CPTs in bacteria have been identified as UPPSs, which synthesize long-chain polyisoprenoid diphosphates (C50–C55) and play an essential role in cell wall biosynthesis [9]. The yeast *RER2* gene has been identified as dehydrodolichyl diphosphate synthase. It catalyzes the synthesis of the long-chain polyprenyl diphosphate that is used as a precursor in the synthesis of dolichol, a mixed *cis, trans*-polyisoprenoid (C75–C95) [10]. In contrast, few CPTs have been reported in plants. Plant genes encoding CPTs from *Hevea brasiliensis* and *Taraxacum brevicorniculatum* are involved in the biosynthesis of natural rubber [12–14], and the *LLA66* gene from *Lilium longiflorum* is involved in microspore development [15]. CPTs also reported to provide the precursors for monoterpene and sesquiterpene biosynthesis in the glandular trichomes of two *Solanum* species [16]. In *Arabidopsis*, there are nine members of the *CPT* gene family [17,18]. One member of this family, *AtCPT1* (At2g23410) may act in the biosynthesis of dolichol [19,20], while another member, *AtHEPS* (*AtCPT6*, At5g58780), was identified as a *Z,E*-mixed heptaprenyl diphosphate synthase [18]. Functions of the other *AtCPT* genes have not yet been characterized. Genomic evidence clearly indicates that *CPT* genes are distributed throughout the plant kingdom.

The triterpene saponins, referred to as ginsenosides, are generally known to be biologically active compounds [21]. Triterpenoid saponins are a class of secondary metabolites (which are mainly produced by various dicotyledonous plant species) that show structural diversity related to their respective biological activities [3]. Thus, functional characterization of the molecular nature of *PgCPT1*, the CPT gene of ginseng, may represent a step forward in our understanding of this medicinal plant. In the present study, molecular cloning and functional characterization of *PgCPT1* have been addressed.

2. Materials and methods

2.1. Plant materials and growth conditions

Korean ginseng (*Panax ginseng* Meyer 'Chun-Poong') seeds were provided by the National Institute of Horticultural and Herbal Science of Rural Development Administration in Eumsung, Korea. The Columbia ecotype (Col-0) of *Arabidopsis thaliana* was used as a model plant. Seeds were sown on 1/2 MS medium (Duchefa Biochemie, Haarlem, The Netherlands) containing 1% sucrose at pH 5.7. Seeds were cold-treated for 2 d and germinated under long-day conditions of 16 h light/8 h dark at 23°C.

2.2. Isolation of PgCPT1 and DNA analysis

A gene encoding CPT from the ginseng expressed sequence tag (EST) library was selected and further crosschecked with the ginseng database constructed by the next-generation sequencing method at Seoul National University. The deduced amino acid sequences were searched for homologous proteins in relevant databases using the BLAST tool of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed using BioEdit software (Version 7.1.9, Ibis Biosciences, Carlsbad, CA 92008). A phylogenetic tree was constructed by the neighbor-joining method, and the reliability of each node was established by bootstrap methods using MEGA6 version 6.06 software.

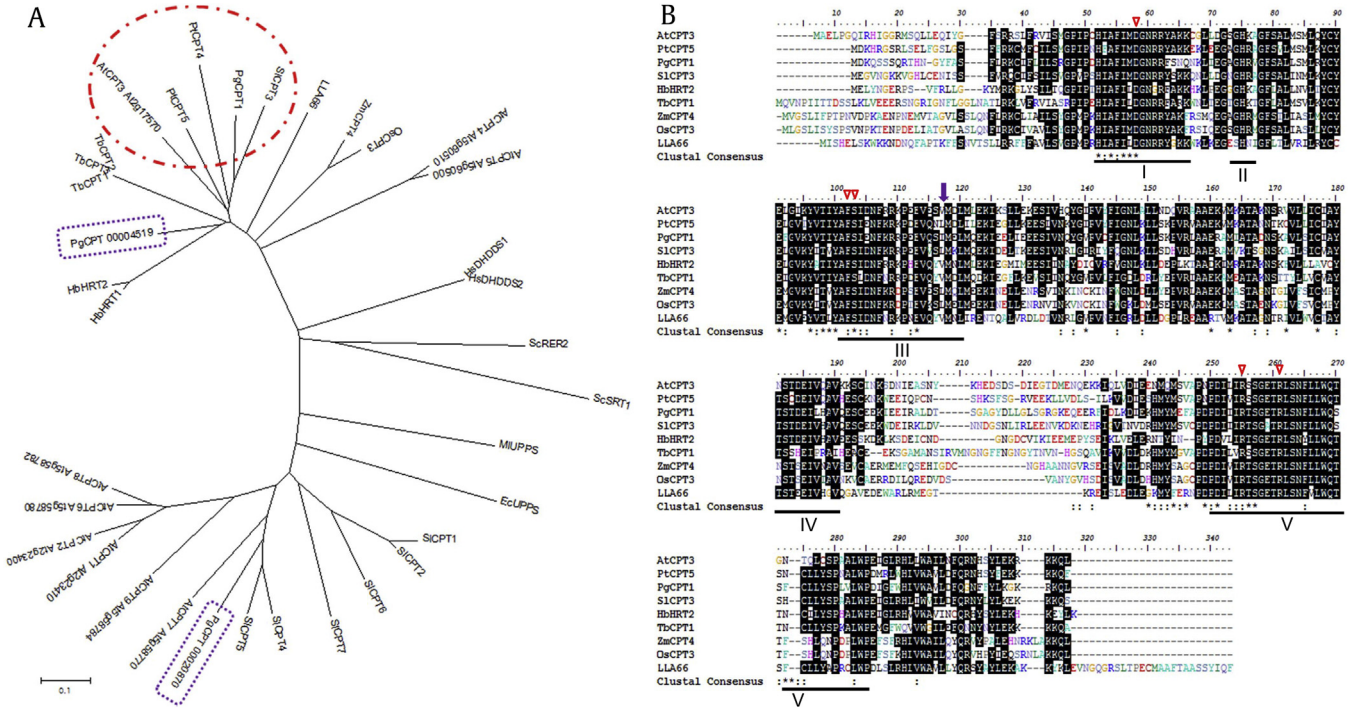

Fig. 1. PgCPT1 proteins are closely related to other CPT proteins. (A) Phylogenetic tree of PgCPT proteins with the closest homologous proteins. The phylogenetic tree was constructed using the ClustalX program (neighbor-joining method). *Sl*, *Solanum lycopersicum; At*, *Arabidopsis thaliana; Tb*, *Taraxacum brevicorniculatum; Pt*, *Populus trichocarpa; Os*, *Oryza sativa; Zm*, *Zea mays; Sc*, *Saccharomyces cerevisiae; Ec*, *Escherichia coli; Ml*, *Micrococcus luteus; Hb*, *Hevea brasiliensis; Hs*, *Homo sapiens; LLA66*, *Lilium longiflorum*. The GenBank accession numbers are: SlCPT1 (NM_001247704); SlCPT2 (JX943884); SlCPT3 (JX943885); SlCPT4 (JX943886); SlCPT5 (JX943887); SlCPT6 (JX943888); SlCPT7 (JX943889); AtCPT1 (NP_565551); AtCPT2 (NP_179921); AtCPT3 (NP_565420); AtCPT4 (NP_200859); AtCPT5 (NP_200858); AtCPT6 (NP_568882); AtCPT7 (NP_200685); AtCPT8 (NP_568883); AtCPT9 (NP_568884); TbCPT1 (JQ991925); TbCPT2 (JQ991926); TbCPT3 (JQ991927); PtCPT4 (XP_002337819); PtCPT5 (XP_002307209); OsCPT3 (NP_001060237); ZmCPT4 (NP_001131688); ScRER2 (P35196); ScSRT1 (NP_013819); EcUPPS (P60472); MlUPPS (O82827); HbHRT1 (BAB71776); HbHRT2 (BAB83522); HsDHDDS1 (NP_995583); HsDHDDS2 (NP_079163); LLA66 (B2BA86). (B) Alignment of PgCPT1 protein with its closest homologs. The underlined domain represents five characteristically conserved regions (I–V) of CPTs. Open triangles indicate residues that are important for catalytic activity and substrate binding. Purple arrow on domain III indicates the starting point of N-terminal deleted *PgNΔCPT1*. Bar represents 0.1 substitutions per amino acid position. PgCPT, *Panax ginseng cis*-prenyltransferase.

2.3. Vector construction and in planta transformation

To visualize subcellular localization patterns of ginseng CPT, genomic DNA sequences of *PgCPT1* (870 nucleotides) with C-terminal and yellow fluorescent protein (YFP) tagging in frame was expressed under the control of the cauliflower mosaic virus 35S promoter. Full-length *PgCPT1* genomic DNA was amplified using primers with *Sal*I and *Sma*I sites: 5′-GC GTC GAC ATG GAT AAA CAG AGT AGT-3′ and 5′-AT CCC GGG TAG TTG CTT CCT TTT ACC-3′. The PCR product of *PgCPT1* was cloned into a modified pCAMBIA1390 vector [22]. These constructs were transformed into *Arabidopsis* Col-0 (CS60000) using *Agrobacterium tumefaciens* C58C1 (pMP90) [23]. Transgenic plants exhibiting a 3:1 Mendelian segregation ratio on hygromycin-containing media were selected for further analysis. For each construct, more than 15 independent T1 lines were obtained and homozygous lines were selected for further analysis.

2.4. RNA isolation and quantitative reverse transcription polymerase chain reaction

Total RNA was isolated from frozen ginseng samples using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Reverse transcription polymerase chain reaction (RT-PCR) was performed in a total reaction volume of 25 μL, consisting of 1−2 μL cDNA, 20 pmol each primer, and AmpONE Taq DNA polymerase (GeneAll, Seoul, Korea) using a Bio-Rad PCR machine: 95°C for 2 min; followed by 28 cycles at 95°C for 30 s; 60°C for 20 s; 72°C for 40 s; and extension at 72°C for 10 min. RT-PCR products were visualized on 1.0% agarose gel. Quantitative PCR was carried out with a reaction volume of 25 μL, using the Thermal Cycler Dice Real-Time PCR System (Takara, Japan), 12.5 μL SYBR Premix Ex Taq (Takara), and *PgCPT1* gene-specific primers: 5′-GGT GCT GGA TAT GAC TTA-3′ (forward) and 5′-CAA CAG GCA AAA CGA ACT-3′ (reverse). The housekeeping gene that encodes *β-actin* (DC03005B05) was used as a control and amplified with the primers: 5′-AGA GAT TCC GCT GTC CAG AA-3′ (left) and 5′-ATC AGC GAT ACC AGG GAA CA-3′ (right). The thermal cycler conditions recommended by the manufacturer were followed: initial denaturation at 95°C for 30 s; followed by 40 cycles at 95°C for 5 s; and 60°C for 30 s. At the end of the PCR, a dissociation curve was generated to evaluate the possibility of undesirable side products. To determine the absolute fold change of each organ, the cycle threshold (Ct) value for *PgCPT1* was normalized to the Ct value for *β-actin* and calculated relative to a calibrator using the formula $2^{-\Delta\Delta Ct}$. Three independent experiments were conducted.

2.5. Abiotic stresses and hormone treatment

Three-week-old ginseng plantlets (Chun-Poong) were subjected to various abiotic stimuli and treatment with two defense-modulating plant hormones. The plantlets were placed on a Petri dish and the roots were dipped in 60 mL of one of the following: 5mM salicylic acid (SA); 0.2mM jasmonic acid (JA); 100μM abscisic acid (ABA); 10mM H_2O_2; and 100mM NaCl. Chilling stress was applied by placing the roots in tap water at 4°C. Treated plantlet samples were gathered at intervals of 1 h, 4 h, 8 h, 12 h, 24 h, and 48 h after treatment. Plant samples were immediately frozen in liquid nitrogen and stored at −70°C.

2.6. Pseudomonas syringae treatment and bacterial growth determination

P. syringae pv. *tomato* DC3000 was cultured in King's B medium containing 50 g/L rifampicin. Bacterial cells used to infect the plants were adjusted to optical density at 600 nm = 0.1 with distilled water and sprayed with 0.01% Silwet L-77. To evaluate bacterial growth, the youngest fully expanded leaves were inoculated with bacterial cells (10^6 cfu/mL) in 10mM $MgCl_2$ and subsequently covered with plastic foil. Infected leaf samples were collected 3 d after infection and immediately ground with 1 mL 10mM $MgCl_2$ using a mortar and pestle. Original sample stocks were diluted 10 times with distilled water. Each sample dilution was dropped onto the surface of a plate containing King's B medium and 50 mg/L rifampicin. The plates were incubated at 28°C for 48 h, following which the newly formed colonies were counted.

2.7. Confocal microscopy analysis

Fluorescence from reporter proteins was viewed with an Olympus Fluoview 500 confocal laser scanning microscope (Olympus, Tokyo, Japan). CFP and YFP were detected using 458/475−525 and 514/>530 nm excitation/emission filter sets, respectively. Fluorescence images were digitized with the Olympus FV10-ASW 4.0 Viewer.

2.8. Functional in vivo complementation assay in the yeast rer2Δ strain

PgCPT1 was cloned into the pYEp352 vector under the native yeast RER2 promoter. PCR amplification of *PgCPT1* was carried out

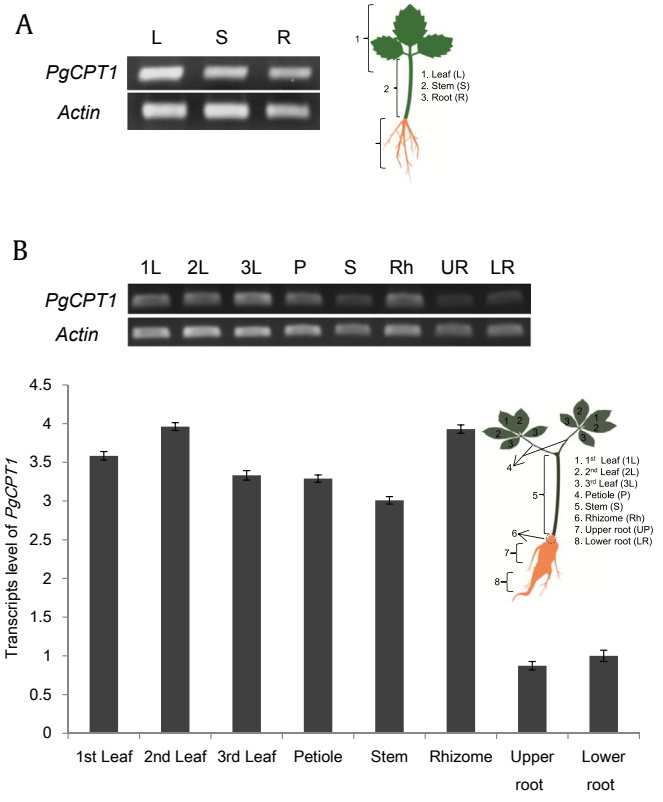

Fig. 2. Organ-specific expression patterns of *PgCPT1*. (A, B) Differential expression patterns of *PgCPT1* in ginseng were evaluated in 3-wk-old plantlets (A) and 2-yr-old plants (B) using reverse transcription polymerase chain reaction (A and B) and quantitative polymerase chain reaction (B, lower panel) with cDNA of the stem, leaf, root, rhizome, and petiole. Exact organ nomenclature for plantlets and 2-yr-old ginseng plants are indicated on the right. The cycle threshold (Ct) value for *PgCPT1* was normalized to the Ct value for *β-actin* and calculated relative to a calibrator sample using the formula $2^{-\Delta\Delta Ct}$. Data represent the mean ± standard error for three independent replicates.

using the following primers with *Sma*I and *Hin*dIII sites (underlined): 5′- AT CCC GGG ATG GAT AAA CAG AGT AGT-3′ (forward) and 5′- GC AAG CTT TTA TAG TTG CTT CCT TTT ACC-3′ (reverse). The PCR product was digested with *Sma*I and *Hin*dIII, and ligated into the pYEp352 vector. The yeast strain YG932 (*rer2Δ* mutant) (*MATα rer2Δ::kanMX4 ade2-101 ura3-52 his3Δ200 lys2-801*) was used in the complementation assay as previously described [4]. The strain was cultured on yeast extract peptone dextrose medium at 23°C. Yeast transformation was performed according to the methods described previously [24] and transformants were selected on uracil selection medium.

2.9. Yeast two-hybrid protein interaction assay

The yeast strain AH109 and vectors provided in the Matchmaker Two-Hybrid System (Clontech, Germany) were used to generate constructs for the interaction study of PgCPT1 with two CPT-like proteins, PgCPTL1 and PgCPTL2. The entire coding sequences of *PgCPT1* and *PgCPTLs* were amplified using the following *Sma*I- and *Sal*I-embedded primers: 5′-at ccc ggg g ATG GAT AAA CAG AGT AGT-3′ (forward) and 5′-tc gtc gac TAG TTG CTT CCT TTT ACC-3′ (reverse) for PgCPT1; and 5′-at ccc ggg g ATG GAT CTT GGA GAT GAG-3′ (forward) and 5′-tc gtc gac TGT ACC ATA GTT TTG TTG-3′ (reverse) for PgCPTL1

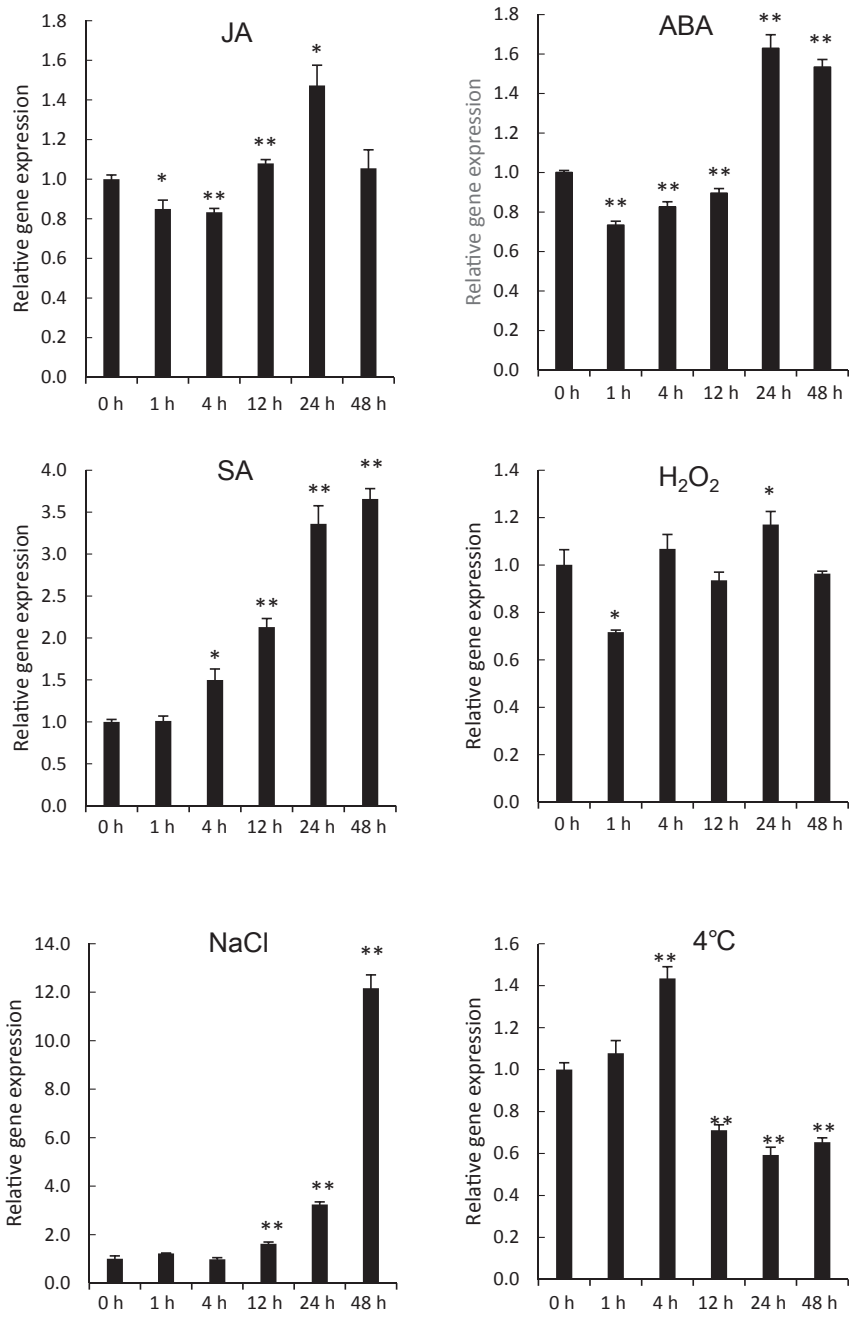

Fig. 3. Temporal expression patterns of the *PgCPT1* gene in response to abiotic stresses. Three-week-old ginseng plantlets were exposed to jasmonic acid (JA; 0.2mM), H_2O_2 (10mM), salicylic acid (SA; 5mM), NaCl (100mM), chilling conditions (4°C), and abscisic acid (ABA; 100μM) for the time intervals indicated. *β-actin* was used as a loading control. The cycle threshold (Ct) value for *PgCPT1* was normalized to the Ct value for *β-actin* and calculated relative to a calibrator sample using the formula $2^{-\Delta\Delta Ct}$. Means for treated samples were significantly different from the control at $^{*}p < 0.05$ and $^{**}p < 0.01$.

and PgCPTL2. PCR products containing *PgCPT1* and *PgCPTLs* were amplified using the aforementioned primer set. Purified *PgCPT1* and *PgCPTLs* were digested with *Sma*I and *Sal*I restriction enzymes and cloned into pGBKT7 and pGADT7, respectively. Plasmids containing *PgCPT1* and *PgCPTLs* were simultaneously transformed into the AH109 strain using the lithium acetate method with modifications [25].

3. Results and discussion

3.1. Isolation and identification of ginseng CPT genes

In order to identify genes coding CPT, EST clones showing amino acid sequence similarity were selected from previously constructed EST libraries of 14-year-old ginseng tissues and hairy roots [26]. Following rapid amplification of cDNA ends PCR, full-length cDNA sequences of *PgCPT1* were obtained. Using the BLAST tool, this *PgCPT1* was searched against the ginseng genome database constructed at Seoul National University, Korea, and two more closely related *PgCPT* genes (*PgCPT 00004591* and *PgCPT 00020870*) were identified (Fig. 1A). These findings suggest that the ginseng genome contains at least one family of three putative genes that are homologous with known *CPT* genes. *PgCPT 0004591* is more closely related to *CPT* genes from the rubber tree (*H. brasiliensis*) and *PgCPT 00020870* is more closely related to *SlCPT4/5* and *AtCPT7* (Fig. 1A). However, the characterization of *PgCPT1* in the present study was more focused. *PgCPT1* is 870 bp in length and encodes 289 amino acids with no introns. This is a typical feature of the most highly homologous *CPT* genes, such as *SlCPT3* and *AtCPT3*, in other plant species [4] (Fig. 1B). Alignment of the deduced amino acid sequence of PgCPT1 with close homologs from *Arabidopsis*, poplar, tomato, the rubber tree, maize, rice, and lily indicates that PgCPT1 also shares the five characteristically conserved regions (I–V) of CPTs [6]. Several important residues such as Asp (D) in Region I that is important for catalytic activity, Phe (F) and Ser (S) in Region III, and two Arg (R) residues in Region V are essential for substrate binding are all conserved in PgCPT1 and depicted with open triangles (Fig. 1B). Interestingly, SlCPT3 is the only one tomato (*Solanum lycopersicum*) CPT protein without any introns or an N-terminal extension (a similar feature of yeast and *Escherichia coli* proteins) [4]. Similarly, ginseng CPT1 also contained no additional residues at the N terminus (Fig. 1B) and no introns, which suggests that this PgCPT1-type of CPT is an ancient form that existed before evolutionary diverged.

3.2. Temporal organ-specific expression patterns of PgCPT1

P. ginseng shows two distinct age-dependent anatomical structures [3]. From 3 wk to 1 yr of age, ginseng plantlets typically have three leaves connected via a single petiole to a stem. From 2 yr of age onwards, ginseng plants have five leaves and the number of petioles increases with the number of years of cultivation. Based on these differences, temporal expression patterns of *PgCPT1* were evaluated. Transcripts of *PgCPT1* were predominantly expressed in the leaves and were observed at lower levels in the stems and roots (Fig. 2A). This mRNA distribution pattern was observed in 2-yr-old ginseng plants, the organs of which were generally well developed. In comparison to the roots, *PgCPT1* transcripts were expressed 4 times and 4.2 times more abundantly in leaves and rhizomes, respectively (Fig. 2B). These data suggest that *PgCPT1* is expressed in all organs, but more predominantly in the leaves and rhizomes than in the roots.

3.3. Differential transcript levels of PgCPT1 in response to abiotic and biotic stresses

A novel gene from *Arabidopsis* containing the CPT domain, *LEW1*, is involved in the response to abiotic stresses, such as drought and osmotic stress [27]. Thus, the possibility of the involvement of *PgCPT1* in the response to various abiotic stresses and phytohormones [28] was analyzed by evaluating the modulated responses of gene transcripts. Relative expression of *PgCPT1* following exposure to several abiotic stresses, such as cold (4°C); NaCl (100mM); H_2O_2 (10mM); ABA (100μM); SA (5mM); and JA (0.2mM) was analyzed using quantitative RT-PCR. Phytohormones comprise both a classical phytohormone, ABA, and small signaling molecules, such as JA and SA were tested. JA and SA are well-known inducible defense modulators that act antagonistically. Among the stimuli tested, SA-specific gradual upregulation of *PgCPT1* was observed (Fig. 3). JA and ABA initially downregulated the mRNA level of *PgCPT1*, and started to upregulated from 24 h after treatment (24 hat). H_2O_2 affected the mRNA level of *PgCPT1* slightly down and up 1 hat and 24 hat, respectively, and did not affect significantly to the rest of treatments. Cold stress downregulated *PgCPT1* once peaked at 4 hat. In contrast, after 12 hat, 100mM NaCl significantly increased *PgCPT1* expression, which peaked at 48 hat. It suggests that *PgCPT1* may play a role in salt-tolerance.

P. syringae is a Gram-negative, rod-shaped bacterium that infects a variety of plants, including *A. thaliana*. Patterns of gene

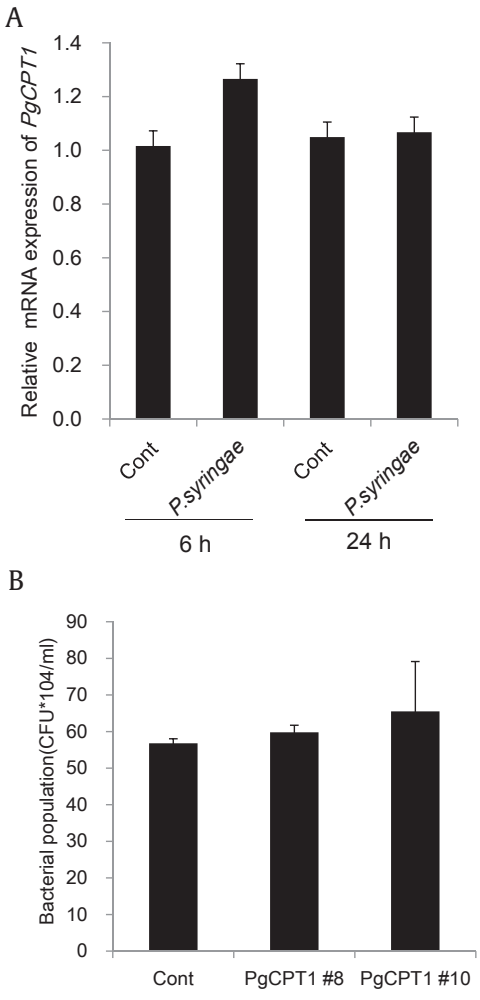

Fig. 4. Temporal expression patterns of the *PgCPT1* gene in response to biotic stress, *Pseudomonas syringae* (A) and *P. syringae* population remained unchanged in *PgCPT1ox* lines compared to Col-0 (B). *P. syringae* for the time intervals indicated. *β-actin* was used as a loading control. Data represent the mean ± standard error for three independent replicates. Means for treated samples were significantly different from the control at $^{*}p < 0.05$ and $^{**}p < 0.01$. Cont, control; PgCPT, *Panax ginseng cis*-prenyltransferase.

expression in infected samples were analyzed at 6 h and 24 h postinfection, to evaluate the response to *P. syringae*. However, no significant patterns of expression and functions were observed (Fig. 4). Taken together, PgCPT1 might function on abiotic salt tolerance instead of biotic stress.

3.4. PgCPT1oxs exhibited no phenotypic differences and localized into cytosol

In order to understand the function of *PgCPT1*, an overexpression construct of full-length (870 bp) *PgCPT1* was generated. Three individual *PgCPT1ox* lines showed no phenotypic differences in fully-grown plant height and floral organs including mature siliques (Fig. 5A). Three individual transgenic lines showed increased transcripts level of *PgCPT1* (Fig. 5B). PgCPT1 displayed cytosol localization in root hair cells and root, respectively (Fig. 5C). Cytosolic localization of PgCPT1 is consistent with that of SlCPT3 in tomato and CPT3 in lettuce [4,29], which suggests the involvement of other accessory proteins that might be required for proper protein targeting and trafficking.

3.5. PgCPT1 partially complements the yeast rer2Δ dolichol mutant

The yeast *rer2Δ* mutant is deficient in dolichol synthesis, as demonstrated by slower growth at temperatures above 30°C and defects in N glycosylation [10]. SlCPT3, the closest homolog to PgCPT1, partially rescues the growth defect of the *rer2Δ* mutant at nonpermissive temperatures [30]. In order to confirm whether PgCPT1 is a functional CPT homolog in dehydrodolichyl diphosphate synthase, full-length cDNA was expressed under the native RER2 promoter. Bacterial *UPPS* that completely complements the *rer2Δ* growth defect [31] was used as a positive control. Introduction of *PgCPT1* into the *rer2Δ* mutant partially suppressed the temperature-sensitive growth defect at elevated temperatures (Fig. 6A). Dolichols are long-chain unsaturated polyisoprenoids with several cellular functions. Dolichol defects can lead to a range of congenital disorders in animals that are typical manifestations of aberrant protein glycosylation. The biological functions of dolichols in plants remain largely unknown. The first evidence of these functions was discovered during a genetic screen for leaf wilting phenotypes in *Arabidopsis* [27]. Further analysis of PgCPT1 focused on drought and osmotic stress may shed light on its biological functions in ginseng plant.

3.6. PgCPT1 interacts with PgCPTL2

Three CPT-like proteins, LEW1 (for leaf wilting1) from *Arabidopsis*, CPTBP from tomato, and CPTL2 from lettuce, have been previously reported [27,30,32]. These all reported to functioning as scaffolding proteins via protein–protein interactions with CPT for proper targeting of proteins in dolichol biosynthesis. One homologous CPT-like protein in humans is the Nogo-B receptor (NgBR). It lacks five conserved motifs, shares weak sequence homology with CPT, and shows < 15% homology at the amino acid level. NgBR represents the first evidence of protein interaction with the human CPT enzyme [11]. Thus, a search of all NgBR and CPT-like homologous genes in the ginseng genome database was conducted, and two coding genes, *PgCPTL1* and *PgCPTL2*, were identified (Fig. 6B). PgCPTL1 shares 54% amino acid sequence identity with LEW1, whereas PgCPTL2 shares 49% amino acid sequence identity with LEW1 (Fig. 6B). Direct *in vivo* protein interaction of PgCPT1 with PgCPTLs was evaluated using a yeast two-hybrid assay. DEF (DEFICIENS) and GLO (GLOBOSA) proteins were used as positive controls [22]. The assay reveals that only PgCPTL2 interacts with PgCPT1 (Fig. 6C), whereas the other close homolog, PgCPTL1, does not. Thus, a two-component enzyme complex system seems to affect dolichol biosynthesis in both ginseng and tomato plants [30]. Similar systems have also been observed in lettuce (*Lactuca sativa*), where an unusual *cis*-prenyltransferase-like 2 (*CPTL2*) is colocalized with CPT3 in the endoplasmic reticulum, with which it interacts

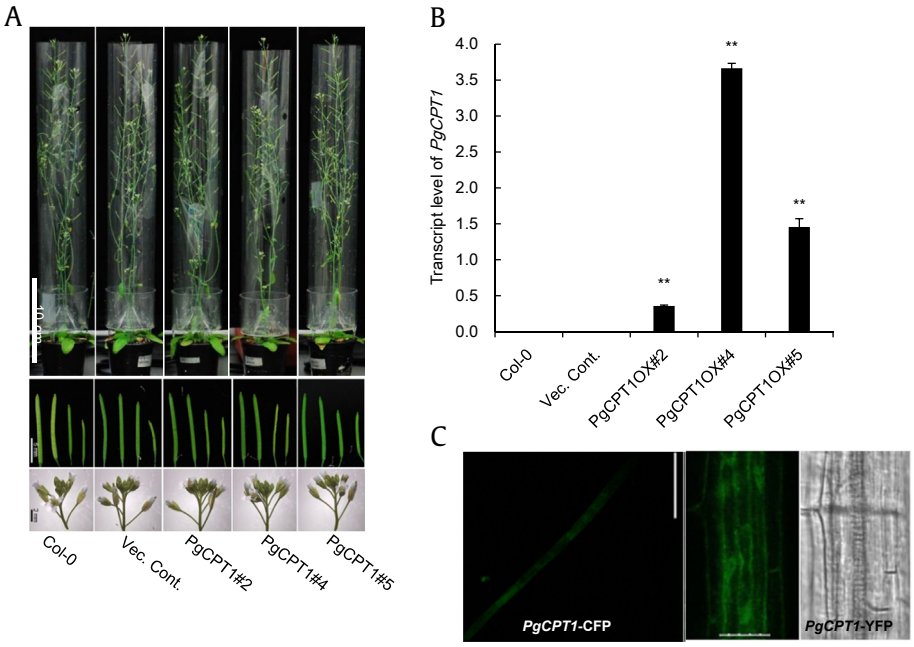

Fig. 5. Overexpression of *PgNΔCPT1* showed no phenotypic defects and localized into cytosol. (A) 50-d-old whole plants show no phenotypic defects in plant height or floral organs compared to the wild type (Col-0) and empty vector control (Vec. Cont.). Scale bars are indicated in each image (B) Overexpression of both *PgNΔCPT1* and *PgCPT1* showed increased transcripts of PgCPT1 mRNA. (C) Fluorescent-tagged *PgNΔCPT1-CFP* and *PgCPT1-YFP* showed cytosol localization. Bar = 50 μm (left) and 25 μm (right) respectively. PgCPT, *Panax ginseng cis*-prenyltransferase.

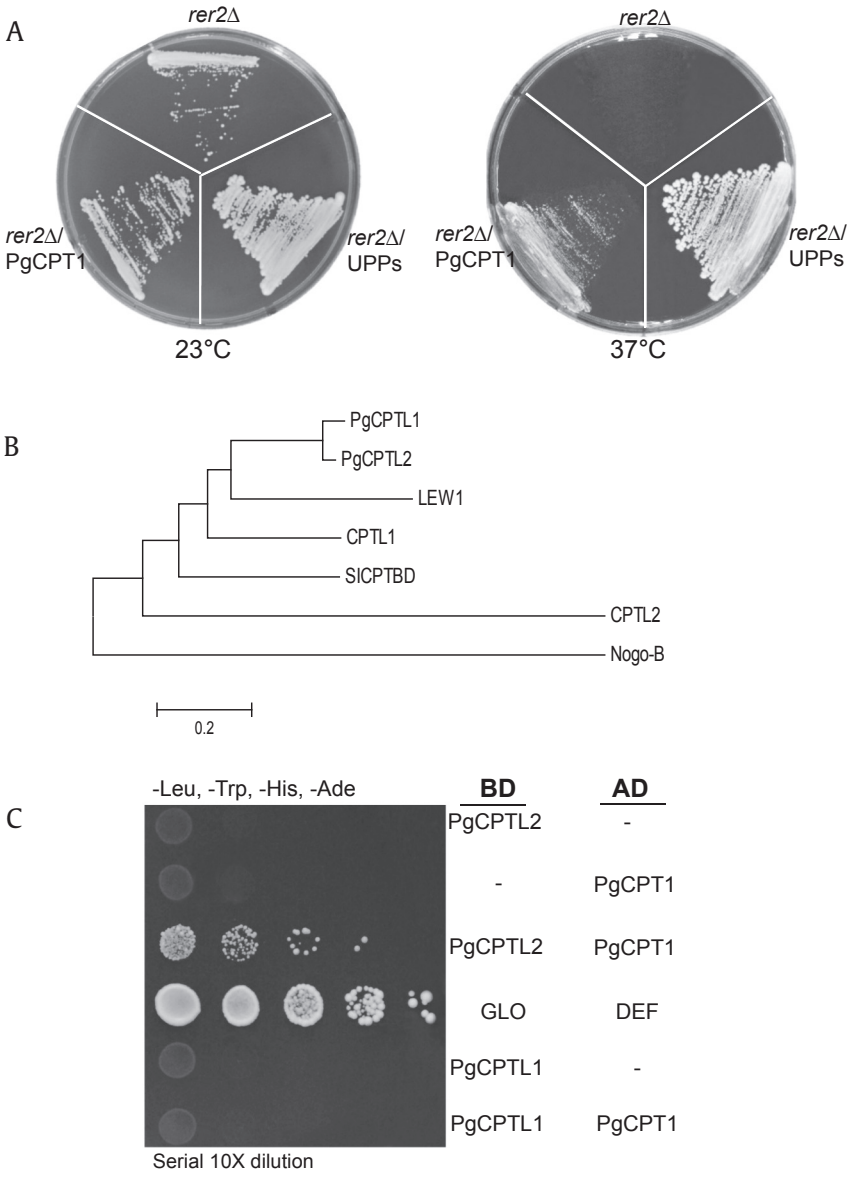

Fig. 6. PgCPT1 partially complement *rer2Δ* and interacts with a human Nogo-B receptor homolog, PgCPTL2. (A) PgCPT1 functionally complemented dolichol-deficient *rer2Δ* yeast mutant. Yeast *rer2Δ* mutant cells were transformed with either *PgCPT1* or the *E. coli UPPS* gene (positive control) under transcriptional control of the native RER2 promoter. Transformed cells were grown on yeast extract peptone dextrose medium at 23°C, and incubated at 23°C and 37°C. (B) The human Nogo-B receptor-like PgCPTL2 is the closest homolog to LEW1 from *Arabidopsis*. (C) Full-coding sequence of genes cloned and expressed in "bait" (pGBKT7) and "prey" (pGADT7) vectors as indicated in the right panel. DEF and GLO proteins, which are known to form heterodimers, were used as a positive control. Cotransformed yeast clones were grown on YSD selective media (-Trp, -Leu, -His, -Ade) and tested for expression of the *LacZ* gene. DEF, ; GLO, ; LEW1, ; PgCPT, *Panax ginseng cis*-prenyltransferase; PgCPTL, ; SICPTDB.

for *cis*-polyisoprene biosynthesis [32]. Our findings suggest that ginseng CPT1 plays an important role in dolichol biosynthesis, and requires an accessory protein for proper function. Considering the fact that both polyprenyl diphosphates and ginsenosides are biosynthesized from the MVA pathway and are competitively regulated by IPP and dimethylallyl diphosphate (DMAPP), it would be interesting to determine whether increased dolichol biosynthesis regulates pathway flux in ginsenoside biosynthesis.

4. Conclusion

Plant isoprenoids comprise a structurally diverse group of compounds that include pigments, hormones, quinones, and sterols, as well as a variety of specialized metabolites that are often restricted to specific genera or families. The C5 compounds, IPP and DMAPP, serve as the precursors of isoprenoids and can be formed either via the plastid-localized 2-C-methyl-D-erythritol 4-phosphate or cytosolic MVA pathways [2,3]. The formation of polyprenyl diphosphates is catalyzed by CPTs, which sequentially add IPPs onto *trans*, *trans*-farnesyl pyrophosphate. Triterpene saponins in *P. ginseng* Meyer (also known as ginsenosides, the majority of which are derived from the mevalonate pathway), are the most widely studied in the pharmaceutical [21] and cosmetic industries. In the present study, the function of a CPT from ginseng was functionally characterized for the first time *in planta* and in a yeast system, which suggests its overexpression do not alter plant growth and development, and may be implicated in dolichol biosynthesis.

Conflicts of interest

All authors have no conflicts of interest to declare.

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