

EXPRESSION OF A SESAME GERANYLGERANYL REDUCTASE cDNA IS INDUCED BY LIGHT BUT REPRESSED BY ABSCISIC ACID AND ETHYLENE

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Abstract

Geranylgeranyl reductase (CHL P) is localized in plastids and catalyzes the reduction of geranylgeranyl diphosphate to phytyl diphosphate, a molecule required for both tocopherol and chlorophyll biosyntheses. To understand the regulation of *Chl P* expression in sesame, a *Chl P* cDNA (*SiChlP*) was isolated and the effects of light and phytohormones on *SiChl P* expression was investigated. The putative *SiChl P* cDNA was 1,633 bp and included a 1,394 bp open reading frame encoding a polypeptide of 465 amino acid residues. *SiChl P* mRNA was expressed most abundantly in leaves and cotyledons, moderately in developing capsules and seeds, but little in flowers, hypocotyls and roots of healthy seedlings and plants. Expression of *SiChl P* in a cotyledon was induced by light, but the level of induction was higher in red light than in far-red or blue light. However, *SiChl P* expression was diminished by dark, ethylene and abscisic acid.

Introduction

Diverse groups of prenyl lipids including chlorophylls and tocopherols are synthesized in the plastids of plant cells. Prenylation of these compounds with the C₂₀-intermediate geranylgeranyl diphosphate (GGPP) is required in order to form their hydrophobic tails for integration into plastid membranes. GGPP, an isoprenoid present in plastid, is synthesized from four molecules of isopentenyl pyrophosphate, which originate in the cytosol (Liu *et al.*, 2005). In tocopherol synthesis in chloroplasts, phytyl diphosphate is transferred to homogentisic acid to form the first tocopherol intermediate (Hirschberg, 1999). The phytyl diphosphate is formed through stepwise reduction of GGPP before condensation with homogentisic acid. In chlorophyll synthesis in etioplasts, however, prenylation of chlorophyllide with GGPP occurs first and is followed by the reduction of GGPP to form phytyl chlorophyll (Rüdiger *et al.*, 1980; Soll *et al.*, 1983). In either case, reduction of GGPP to phytyl diphosphate is catalyzed by GGPP reductase (CHL P) (Tanaka *et al.*, 1999).

Tocopherols are lipid soluble antioxidants known collectively as vitamin E. Four isoforms of tocopherols, α -, β -, γ - and δ -tocopherol, are synthesized in plants and other photosynthetic organisms. The isoforms differ by the numbers and positions of methyl substituents on the aromatic rings of the molecules (Hess, 1993). The major tocopherols found in human diets are γ - and α -tocopherol (Sheppard & Pennington *et al.*, 1993). Natural sources of α -tocopherol (*R,R,R*- α -tocopherol) are the most bio-potent form of

vitamin E (Machilin, 1991). Clinical and epidemiological evidence suggest that vitamin E decreases the risk of cardiovascular disease and cancer, strengthens the immune system, and prevents or slows various chronic degenerative diseases and aging (Pryor, 2000; Venkateswaran *et al.*, 2002). Sesame oil contains about 528 $\mu\text{g/g}$ of total tocopherols (Cooney *et al.*, 2001). Thus, consumption of tocopherol through sesame seeds and oils could provide health promoting effects (Cooney *et al.*, 2001).

Seed oils of oilseed crops such as sesame and soybean are important sources of vitamin E in the human diet. Due to the beneficial effects of vitamin E, tocopherol content in seeds is becoming one of the major traits for improvement through conventional breeding and metabolic engineering (Shintani & DellaPenna, 1998). The immediate tocopherol intermediate is formed through a condensation of homogentisic acid (HGA) and a phytol sidechain. Therefore, to increase total tocopherol content in crops through genetic manipulation, genes for the key enzymes in the tocopherol biosynthesis pathway such as p-hydroxyphenyl pyruvate dioxygenase (HPPDase), CHL P, and phytyl/prenyl transferase should be identified and characterized. Recent cloning of tocopherol phytyltransferases in *Synechocystis* and *Arabidopsis* will promote cloning of the homologues in other species (Schledz, 2001; Savidge *et al.*, 2002).

CHL P has been investigated in detail in a few plant species such as tobacco (Tanaka *et al.*, 1999) and *Arabidopsis* (Keller *et al.*, 1988). Soybean, ice plant and several photosynthetic bacterial *Chl P* sequences have also been identified. A few studies in tobacco and peaches have reported that expression of *Chl P* was induced by light (Tanaka *et al.*, 1999; Giannino *et al.*, 1988). However, there has been little research conducted on the effects of light quality and phytohormones on *Chl P* expression. Thus, this study was conducted to characterize the effects of light and phytohormones on the expression of *Chl P* from sesame.

Materials and Methods

Bacterial strains and plant materials: *Escherichia coli* strains XL1 Blue (Stratagene, USA), JM109 (Promega, USA), and Qiagen EZ (Qiagen, USA) were used for cloning according to standard techniques (Sambrook & Russell, 2001) homologues in other species (Schledz, 2001; Savidge *et al.*, 2002).

Sesame (*Sesamum indicum* L. cv. Dasak) and soybean (*Glycine max* (L.) Merrill. cv. Iksan) plants were grown in a greenhouse and in the experimental field at Chonbuk National University, Jeonju, Korea. Leaves and flowers of sesame were collected from healthy plants grown in the field. Developing capsules were collected 14 and 28 days after flowering (DAF), and developing seeds were separated from the capsules. Sesame seeds were germinated for seven days, and the seedlings were dissected into cotyledons, hypocotyls and roots. Soybean leaves were collected from healthy plants grown in a greenhouse. All the samples were ground into fine powder in liquid nitrogen and kept at -70°C until use.

Cloning and DNA sequence analysis: A soybean *Chl P* probe was generated by amplification using bioinformatics on the known soybean *Chl P* sequence. The sequence of soybean *Chl P* was obtained from the GenBank database (NIH, USA), and forward (5'-GGCTCGACATGAAGTCCATAGCC-3') and reverse (5'-TAAGATCTTCATACGT TAAGTTTGTTTCATCATCTCCC-3') primers targeted to the 5' and 3' ends of the ORF were synthesized. A soybean *Chl P* sequence was amplified by polymerase chain reaction (PCR) with the above primer pair and soybean genomic DNA in a 25 μl reaction mixture consisting of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl_2 , 200 mM primer, 200

μM dNTP, 1 unit *Taq* DNA polymerase (TaKaRa, Japan), and 10 ng template DNA. Amplifications were performed in a thermal cycler (Hybaid, UK) with an initial denaturation at 95°C for five min, 35 cycles of 50 sec at 95°C, one min at 50°C, one min at 72°C, and a final extension at 72°C for five min. PCR products were separated by electrophoresis on 1.5% w/v agarose gels in $1 \times \text{TAE}$ buffer (Sambrook & Russell, 2001). The specifically amplified 1.5 kb DNA fragment was cloned into pGEM-T Easy vector (Promega, USA). The nucleotide sequence of the resulting clone, *pGmChl P*, was determined as described (Sambrook & Russell, 2001) and confirmed by comparing against the sequence deposited in GenBank (NCBI, USA). The sequence of *pGmChl P* showed 99% identity to that of soybean *Chl P* (Accession no. AAD28640) and the clone was used as a probe for screening a sesame cDNA library. A cDNA library of sesame seed was constructed and screened in order to isolate a *Chl P* clone. About 2×10^5 pfu of the cDNA library was plated onto NZY agar plates and the plaques were transferred to nylon membranes (Hybond-N⁺, Amersham, UK). The DNA on the membrane was fixed using a UV crosslinker (GS Gene Linker, BioRad, USA). The membrane was used for hybridization with the *GmChl P* probe. Labeling, hybridization, washing and detection of sesame *Chl P* cDNA were conducted with AlkPhos Direct system (Amersham Pharmacia Biotech, UK) according to the manufacturer's recommendations. From the screening, one secondary positive clone was isolated and designated *SiChl P*. The insert DNA of *SiChl P* was subcloned into pBluescript SK (+), and the nucleotide sequences of both strands were determined by the dideoxy chain termination method (Sanger *et al.*, 1977). Nucleotide and deduced amino acid sequence analyses were performed using DNASIS (HITACHI, USA) and the programs and databases offered by the National Center for Biotechnology Information (NCBI, USA) and European Bioinformatics Institute (EBI, UK). Multiple sequence analysis was performed using the program AliBee (GeneBee, SU).

Light and phytohormone treatments: Sesame seeds were germinated either in the light or in the dark for one week. Phytohormone treatments were performed with light-germinated seedlings. One-week-old light-germinated seedlings were separated into the cotyledon and root and then they were immersed in sterile water, 100 μM abscisic acid (ABA) or 0.5 mM ethylene in the dark for 24 h at 25°C. Light treatment was conducted using dark-germinated seedlings. The dissected cotyledons of one-week-old dark-germinated seedlings were immersed in sterile water and kept in the dark or treated with white light, red light (50 $\mu\text{mol}/\text{m}^2/\text{s}$), far-red light (50 $\mu\text{mol}/\text{m}^2/\text{s}$), or blue light (50 $\mu\text{mol}/\text{m}^2/\text{s}$) for 24 h at 25°C.

Southern and Northern blot analysis: Southern blot analysis was carried out with genomic DNA as described (Sambrook & Russell, 2001). Genomic DNA digested to completion with restriction enzymes (*Bgl*III, *Nde*I, or *Bgl*III + *Nde*I) was separated by agarose gel electrophoresis and transferred onto a nylon membrane (Hybond-N⁺, Amersham, UK). The membrane was used for hybridization with the *SiChl P* probe. Labeling, hybridization, washing and detection of signals were conducted with the AlkPhos Direct system (Amersham Pharmacia Biotech, UK) according to the manufacturer's recommendations. Total RNA was extracted using the TRI reagent procedure (MRC, USA). For Northern blot analysis, total RNA (20 μg) was denatured, separated on a 1.2% formaldehyde gel, and transferred onto a nylon membrane (Hybond-N⁺, Amersham, UK). The membrane was hybridized with the *SiChl P* probe labeled with alkaline phosphatase, and the signal was detected as for Southern blots.

1	CT	TAA	CTA	AAA	TCA	CCC	CCC	ATG	GCT	TCC	ATC	GCC	CTC	AAA	ACC	TTC	GTC	GGC	CTC	CGC	CAG	TGG	ACG	CCG	71	
72	GAA	AAT	AAC	GCT	ATA	GTT	CTC	TCC	AAG	CCC	ATC	GCC	ACC	ACC	ACC	TTG	CCC	TAC	CGT	AGG	TTA	CGC	GTA	AAT	143	
18	E	N	N	A	I	V	L	S	K	P	I	A	T	T	T	L	P	Y	R	R	L	R	V	N	41	
144	GCC	TCG	AAA	TCC	AGC	CCC	CGA	GTC	ACT	GGG	CGC	ACT	CTT	AGA	↓	GTC	GCT	GTG	GTT	GGG	GGT	CGT	GCT	GGT	215	
42	A	S	K	S	S	P	R	V	T	G	R	N	L	R	V	A	V	G	G	G	P	A	G	65		
216	GGC	CGC	CCC	GGG	GAG	ACA	CTG	GCC	AAG	GGA	GGC	ATC	GAG	AGC	TTT	CTC	ATC	GAA	CGG	AAA	TTG	GAC	AAC	TGC	287	
66	G	A	A	A	E	T	L	A	K	G	G	G	I	E	T	F	L	I	E	R	K	L	D	N	C	89
288	AAG	CCC	TGT	GGC	GGC	GCA	ATC	CGT	CTA	TGC	ATG	CTC	GGG	GAA	TTT	CGC	CTT	CCG	CTG	GAC	ATC	ATT	GAC	CGT	359	
90	K	P	C	G	G	A	I	P	L	C	M	V	G	E	F	D	L	P	L	D	I	I	I	D	R	113
360	AGA	GTG	ACC	AAA	ATG	AAG	ATG	ATT	TCG	CGC	TCT	AAT	GTT	GCC	GTG	GAC	AIT	GGC	CAA	AGC	CTG	AAG	CCC	CAC	431	
114	R	V	T	K	M	K	M	I	S	P	S	N	V	A	V	D	I	G	Q	T	L	K	P	H	137	
432	GAG	TAT	ATT	GCG	ATG	GTC	CGC	GCG	GAA	GTA	CTC	GAT	GCT	TAC	CTC	CGC	GAC	CGC	TCC	GAC	GCC	GGA	GCC	503		
138	E	Y	I	G	M	V	R	R	E	V	L	D	A	Y	L	R	D	R	A	S	D	A	G	A	161	
504	ACT	GTC	ATC	AAC	GGC	CTC	TTC	TTG	AAA	ATG	GAC	CTG	CCC	CAG	TCC	AAG	AAC	GCG	CCG	TAC	GTT	TTA	CAC	TAT	575	
162	T	V	I	N	G	L	F	L	K	M	D	L	P	Q	S	K	N	A	P	Y	V	L	H	Y	185	
576	ACC	GAG	TAC	AAC	GCG	AAA	ACA	GGG	AGC	GCT	GGC	GAG	ARG	AGC	ACT	ATG	GAA	GTA	GAC	GCC	GTC	ATT	GGC	GCC	647	
186	T	D	Y	N	A	K	T	G	S	A	G	E	K	K	T	M	E	V	D	A	V	I	G	A	209	
648	GAG	GGC	GCA	AAC	TCC	CGC	GTC	GCA	AAA	GGC	ATC	AAC	GCC	GGC	GAC	TAC	GAT	TAC	GCC	ATT	GCC	TTT	CAA	GAG	719	
210	D	G	A	N	S	R	V	A	K	G	I	N	A	G	G	D	Y	D	Y	A	I	A	F	Q	A	233
720	CGC	ATC	AAA	ATC	TCA	GAT	GAA	AAA	ATG	AAG	TAC	GAA	AAT	TTG	GCA	GAA	ATG	TAC	GTC	GGT	GAA	GAT	GTC	791		
234	R	I	K	I	S	D	E	K	M	K	Y	E	N	L	A	E	M	Y	V	G	E	D	V	257		
792	TGG	CGT	GAT	TTT	CAC	GGT	TGG	GTT	TTC	CCC	AAA	TGC	GAC	CAC	GTC	GCC	GTC	GGT	AGC	ACG	GTG	ACC	CAC	863		
258	S	P	D	F	Y	G	W	V	F	P	K	C	D	H	V	A	V	G	T	G	T	V	T	H	281	
864	AAA	GGC	GAC	ATC	AAG	AAA	TTC	CAA	CTC	GCG	ACT	AGA	CTC	CGC	GCC	CGA	GAC	AAA	ATC	GAA	GGG	GGG	AGA	ATC	935	
282	K	G	G	D	I	K	K	F	Q	L	A	T	R	L	R	A	R	D	K	I	E	G	R	I	305	
936	ATC	CGC	GTG	GAA	GCG	CAC	CCC	ACT	CGT	GAA	CAC	CGG	CGA	AAG	CGA	GTC	CTC	GAC	AGG	GTT	CGC	CTG	GTC	1007		
306	I	R	V	E	A	H	P	I	P	E	H	P	R	P	K	R	V	L	D	R	V	A	L	V	329	
1008	GGC	GAC	CCC	CGC	GGG	TAT	GTT	ACA	AAA	TGC	TCC	GGC	GAG	GGG	ATA	TAC	TTT	CGC	GCG	AAG	AGC	GGG	CGG	ATG	1079	
330	G	D	A	A	G	Y	V	T	K	C	S	G	E	G	I	Y	F	A	A	K	S	G	R	M	353	
1080	TGC	GCT	GAG	GCG	ATA	GTG	GAG	GGA	TGC	GAG	AGG	AGG	AGG	AGG	ATG	GTG	GAG	GAG	AGT	GAT	TTG	AGG	GTG	TAT	1151	
354	C	A	E	A	I	V	E	G	S	E	N	G	K	R	M	V	E	E	S	D	L	R	V	Y	377	
1152	TTG	GAG	AAG	TGG	GAC	AAG	ACG	TAT	TGG	CCG	ACG	TAC	AAG	GTT	TTG	GAT	ATC	CTG	CAG	AAG	GTT	TTT	TAC	CGA	1223	
378	L	E	K	W	D	K	T	Y	W	P	T	Y	K	V	L	D	I	L	Q	K	V	F	Y	R	401	
1224	TGC	AAT	CCG	GCG	AAG	GAG	GCA	TTC	GTG	GAG	ATG	TGC	GCC	GAC	GAG	TAT	GTG	CAG	AAG	ATG	ACC	TTC	GAC	AGC	1295	
402	S	N	P	A	K	E	A	F	V	E	M	C	A	D	E	Y	V	Q	K	M	T	F	D	S	425	
1296	TAT	TTG	TAC	AAG	CGG	GTC	GTG	CCC	GGG	AAC	CCG	TTG	GAG	GAT	TTG	AAG	TTG	GCT	GTG	AAC	ACC	ATT	GGG	AGC	1367	
426	Y	L	Y	K	R	V	V	P	G	N	P	L	E	D	L	K	L	A	V	N	T	I	G	S	449	
1368	CTG	GTA	AGG	GCT	AAT	GCG	CTG	AGG	AAG	GAG	ATG	GAG	AGG	CTT	AGT	GTA	TAA	GCG	GGT	GGA	TGA	CGC	GGT	TGG	1439	
450	L	V	R	A	N	A	L	R	K	E	M	E	K	L	S	V								465		
1440	AAG	GTA	TTG	ATC	AGT	AGT	TGA	TCA	CTT	GTT	TGT	GGG	AGG	CAA	TTG	ATT	TAT	ACG	AGG	AAG	GTT	GTG	GAA	TTC	1511	
1512	CTC	GAA	TGA	GAG	AGT	TGT	ACT	TTC	CTA	TAT	GTT	AAT	TCT	ACC	TAT	CTG	TCT	GAA	ATA	CTT	CTG	TTG	TAG	TTG	1583	
1584	AAG	CGT	GTA	AGA	TAA	AGC	TAT	GTT	GAT	TAG	AAC	AAA	AAA	AAA	AAA	AAA	AAA							1633		

Fig. 1. Nucleotide and deduced amino acid sequences of the sesame *Chl P* cDNA clone, *SiChl P* (GenBank Accession Number GQ860303). The arrow indicates the predicted protease cleavage site of the predicted signal peptide. The boxed residues are in a consensus sequence of the V/IXGX₁₋₂GXXGXXXG/A motif that stabilizes binding of a nucleotide cofactor, FAD or NAD(P).

Results

Structural characteristics of SiChl P cDNA: The 1,633-bp nucleotide and deduced amino acid sequences of *SiChl P* (NCBI GenBank Accession Number GQ860303) are shown in Fig. 1. The nucleotide sequence of *SiChl P* includes a 1,394 bp ORF and untranslated 5' and 3' flanking sequences, including a poly (A) tail. The ORF encoded a 465-amino acid polypeptide with a predicted molecular mass of 51,379 Da and a theoretical pI of 9.01. In the signal peptide sequence, there were six residues each of serine and threonine, constituting 22% of the signal peptide residues, but there was only one glutamate and no aspartate (Fig. 1). The predicted molecular mass of the transit peptide was 6,036 Da. The resulting mature polypeptide consisted of 405 amino acid residues with a calculated molecular mass of 45 kDa.

The deduced 537-amino acid sequence of SiCHL P showed 91, 88, and 84% identities to tobacco (NtCHL P, CAA07683), soybean (GmCHL P, AAD28640) and Arabidopsis (AtCHL P, CAA74372), respectively. However, the SiCHL P sequence showed less than 65% identity to bacterial CHL P sequences. Among the bacterial CHL P polypeptides, the *Synechocystis* CHL P shared the highest identity, 65%, with SiCHL P (Fig. 2). A striking difference between plant and bacterial CHL Ps is the lack of a transit peptide sequence at the amino terminus in bacterial CHL Ps (Fig. 2A). The V/IXGX₁₋₂GXXGXXXG/A motif that binds NAD(P) or FAD is conserved in both plant and

bacterial CHL Ps. A phylogenetic tree based on multiple comparisons of CHL P polypeptide sequences clusters SiCHL P with CHL P of tobacco (Fig. 2B).

Southern blot was probed to estimate the number of sequences homologous to *SiChl P* cDNA present in the sesame genome. The blot was washed at high stringency and showed the presence of one or two major and minor bands (Fig. 3).

Tissue-specific expression of SiChl P: Expression of *SiChl P* was investigated in the different tissues of the sesame plant and germinating seedlings. A 1.6-kb *SiChl P* mRNA was detected most abundantly in leaves and cotyledons, moderately in developing capsules and seeds, but little in flowers, hypocotyls or roots of healthy plants and seedlings (Fig. 4).

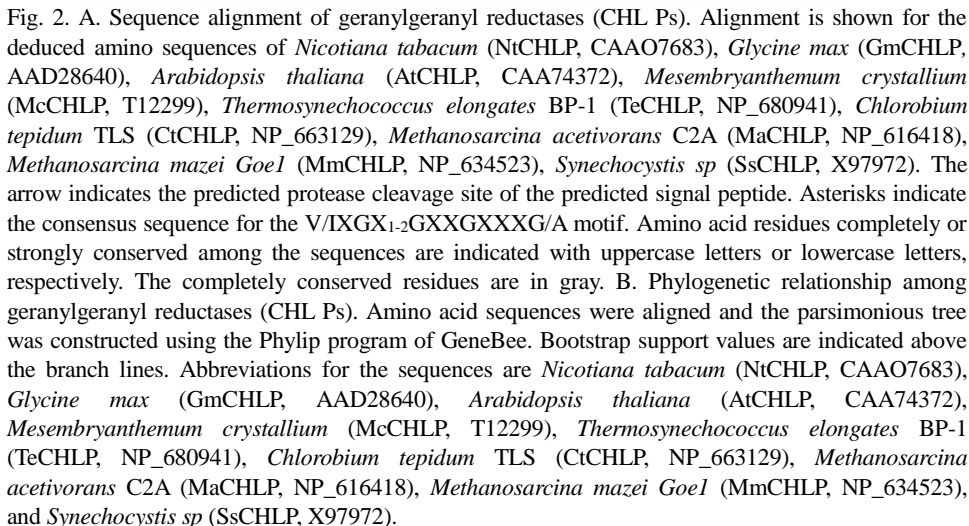
Effect of light and phytohormone on SiChl expression: *SiChl P* mRNAs in the cotyledon were induced by light but repressed by dark. Red light was more effective in the induction of *SiChl P* expression than was blue or far-red light (Fig. 5A). Induction of *SiChl P* mRNA in the cotyledon by light was abolished by ABA and ethylene. However, *SiChl P* mRNAs in the root were not induced by light, ABA or ethylene (Fig. 5B).

Discussion

CHL P catalyzes the reduction of GGPP to phytyl diphosphate in the chloroplast. As plant CHL Ps are localized in chloroplasts, the precursor protein should contain a targeting sequence at the N-terminal region for proper localization of chloroplast proteins synthesized in the cytoplasm (Keegstra & Cline, 1999). The N-terminal sequence of the deduced SiCHL P polypeptide contains a sequence characteristic of transit peptides found in precursor proteins targeted to the chloroplast. The neural network program ChloroP (Emanuelsson *et al.*, 1999) predicts that the transit peptide consists of 55 amino acids with the cleavage site located between A55 and A56 in the sequence NLR → VAV. Despite their common function, transit peptide domains from various precursor proteins share minimal sequence identity, so no consensus sequences have been established. Similar to most chloroplast transit peptides, the transit peptide predicted from *SiChl P* is rich in serine and threonine but deficient in acidic amino acids (Keegstra *et al.*, 1989).

Geranylgeranyl reductase belongs to the family of oxidoreductases. Oxidoreductases contain a nucleotide cofactor-binding domain stabilizing the interaction of the β -strand and α -helix connected by a short loop in which the ligand binding domain is located (Kleiger & Eisenberg, 2002). A typical motif commonly found in oxidoreductases, V/IXGX₁₋₂GXXGXXXG/A, is also present in the N-terminus of the mature SiCHL P polypeptide.

Chl P sequences have been identified from limited plant species and a few photosynthetic bacterial species (Tanaka *et al.*, 1999; Keller *et al.*, 1988; Adlasee *et al.*, 1996). Comparisons of the nucleotide and deduced amino acid sequences of SiCHL P with the *Chl P* sequences in the database revealed a high degree of similarity to the plant *Chl Ps*. A phylogenetic tree based on the multiple comparisons of CHL P polypeptide sequences clusters SiCHL P with CHL P of tobacco, indicating that the closest evolutionary relationship to sesame CHL P is tobacco CHL P. The structural features revealed from the sequence analysis suggest putative identity of the clone as *SiChl P*. In contrast to the bacterial CHL Ps, the SiCHL P contains an amino-terminal extension that resembles a plastid transit peptide sequence, indicating the possible plastid localization of the enzyme.



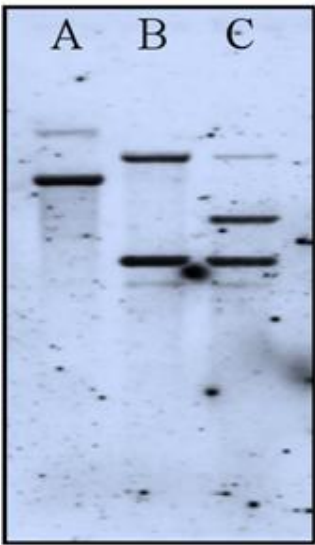


Fig. 3. Southern blot analysis of sesame genomic DNA digested with restriction enzyme *Bgl*II (A), *Nde*I (B), or *Bgl*II and *Nde*I (C). Ten micrograms of sesame genomic DNA digested with the restriction enzymes was separated on a 0.8% (w/v) agarose gel and transferred to a nylon membrane, then hybridized with alkaline phosphatase-labeled *SiChl P* insert DNA.

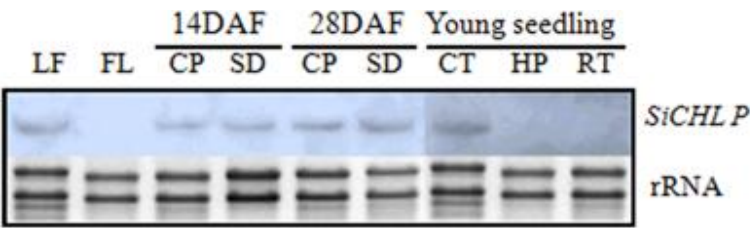


Fig. 4. Expression of *SiChl P* mRNA in the tissues of sesame plant. Leaf (LF), flower (FL), capsule (CP) and seed (SD) from 14 and 28 days after flowering (DAF). Cotyledon (CT), hypocotyl (HP), and root (RT) of seven-day-old seedlings. Twenty micrograms of total RNA was resolved in a 1.0% (w/v) agarose/formaldehyde gel, transferred to a nylon membrane, then hybridized with alkaline phosphatase-labeled *SiChl P* insert DNA. Ethidium bromide-stained rRNA bands as an indicator of equal loading (rRNA).

Because cultivated sesame is a cytogenetic diploid, the Southern blot result indicated that *SiChl P* is most probably present as a single gene with a remote sequence sharing low homology. Appearance of the one major band by *Bgl*II but two by *Nde*I digestion may indicate the presence of intron(s) containing *Nde*I-recognition sequences.

The expression patterns of *SiChl P* are in good agreement with the localization and roles of the CHL P enzyme in plants. The CHL P enzyme is localized in plastids and catalyzes the reduction of geranylgeranyl diphosphate to phytyl diphosphate, a molecule required for both tocopherol and chlorophyll biosyntheses (Tanaka *et al.*, 1999; Grasses *et al.*, 2001). Thus, higher expressions in developing seeds and green tissues supports similar

roles for CHL *P* in sesame. *Chl P* mRNA is expressed at higher levels during de-etiolation in *Arabidopsis* and during differentiation of chromoplasts in pepper (Keller *et al.*, 1988).

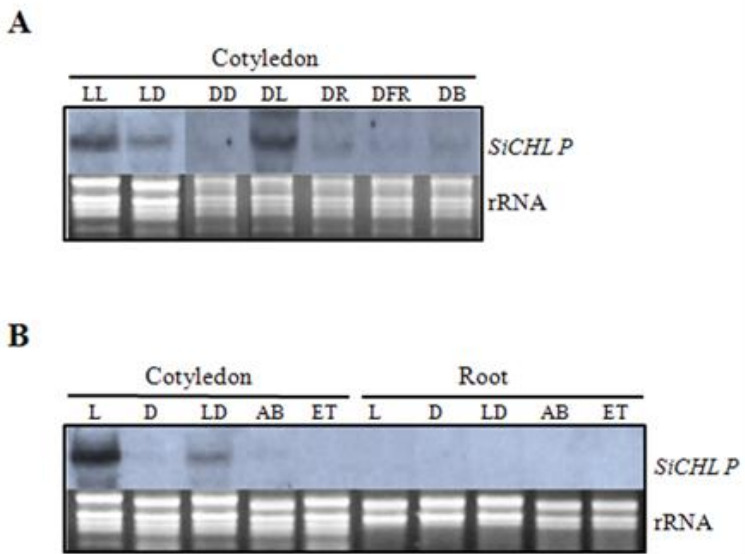


Fig. 5. Effects of light (A) and phytohormones (B) on *SiChl P* expression. A. Cotyledons dissected from one-week-old dark-germinated seedlings were immersed in sterile water and kept in the dark (DD) or treated with white light (DL) at 50 $\mu\text{mol}/\text{m}^2/\text{s}$, red light (DR) at 50 $\mu\text{mol}/\text{m}^2/\text{s}$, far-red light (DFR) at 50 $\mu\text{mol}/\text{m}^2/\text{s}$, or blue light (DB) at 50 $\mu\text{mol}/\text{m}^2/\text{s}$ for 24 h at 25°C, respectively. Cotyledons from one-week-old light-germinated seedlings which were immersed in sterile water and kept in white light (LL) or transferred to the dark for 24 h (LD) are also presented for comparison. B. For phytohormone treatments, the cotyledons and roots of one-week-old light-germinated seedlings were used. The cotyledons and roots from light-geminated seedlings were immersed in sterile water (LD), 100 μM abscisic acid (AB) or 0.5 mM ethylene (ET) in the dark for 24 h at 25°C, respectively. The cotyledons and roots of one-week-old seedlings germinated in the light (L) or dark (D) before phytohormone treatments are also presented for comparison. Twenty micrograms of total RNA was resolved in a 1.0% (w/v) agarose/formaldehyde gel, transferred to a nylon membrane, then hybridized with alkaline phosphatase-labeled *SiChl P* insert DNA. Ethidium bromide-stained rRNA bands as an indicator of equal loading (rRNA).

Expression of *SiChl P* was stimulated by light in cotyledons, but the expression was repressed by dark. Although *SiChl P* was induced by red, far-red and blue light, red light was most effective in the induction of *SiChl P* expression. Light signals adjust plant growth and development in the prevailing environmental conditions (Casal *et al.*, 2004). *Chl P* mRNA of peaches (Giannino *et al.*, 1988) and tobacco (Tanaka *et al.*, 1999) are induced by light. Plants tend to adapt the structure of their photosynthetic apparatus and pigment composition to light quality and quantity (Buschmann *et al.*, 1978). Although regulation of development by light is mediated by photoreceptors in higher plants, different wavelengths of light activate different photoreceptors (Seo *et al.*, 2004). *Arabidopsis* has five phytochromes (phyA through phyE), the photoreceptors that perceive red and far-red light (Quail *et al.*, 1995). All members of the phytochrome family and chlorophylls are regulated by red light, which rapidly establishes a high

proportion of phytochrome active forms (Seo *et al.*, 2004). Far-red light activates the high-irradiance response mode, which requires specific domains of the phyA molecule of *Arabidopsis* phytochromes (Yanovsky & Kay, 2002). Also, through studies of phytochrome A-deficient (*phyA*) mutants, it is known that *Arabidopsis Chl P* expression is up-regulated by phyA (Kuno *et al.*, 2000). Blue light activates cryptochromes (Cashmore *et al.*, 1999; Lin & Shalitin, 2003) and phototropins (Kasahara *et al.*, 2002), which are specific blue light photoreceptors. Thus, it is probable that induction of *SiChl P* expression by light is mediated by the phytochrome family. However, convincing evidence for this assumption should be obtained from further investigation on the role of phytochromes in *SiChl P* expression in sesame.

Ethylene is required in the transduction pathway activated during injury. Ethylene plays an important role in mediating the wound responses of tomato plants (O'Donnell *et al.*, 1996). In the ABA-dependent pathway, ABA is produced upon drought and cold stress with subsequent induction of expressions of various subsets of downstream genes (Uno *et al.*, 2000; Yoshida *et al.*, 2002). In peach leaves, *PpChl P* expression is diminished by cold stress and wounding (Giannino *et al.*, 1988). The expression of *SiChl P* was not induced by ethylene or ABA treatment in cotyledons and roots. Therefore, this result indicates that *SiChl P* is negatively regulated by ethylene, which plays an important role in mediating the wound response, and a subset of genes regulated by ABA in an ABA-dependent pathway.

Taken together, the expression of *SiChl P*, which is most highly expressed in the leaves and developing seeds, is induced by light but repressed by dark, ABA and ethylene. This information can be used for further studies on the regulation of chlorophyll and tocopherol syntheses by light and phytohormones in sesame.

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