Journal of Plant Biology, September 2004, 47(3) : 203-209

Molecular Cloning and Characterization of the *psb*L and *psb*J Genes for Photosystem II from *Panax ginseng*

Ki-Yeol Yoo, Dae-Sung Park, and Gun-Sik Tae*
Department of Biology, College of the Advanced Science, Dankook University, Chungnam 330-714, Korea

We have cloned and characterized two genes for Photosystem II from chloroplasts of *Panax ginseng*. These genes, *psb*L and *psb*J, comprise 117 and 123 nucleotides, respectively. When compared with monocots, dicots, or liverwort, the overall amino acid sequence identity of the former is >97%, whereas that of the latter is approximately 95 to 100%. Southern blot analysis revealed that a single copy of each gene exists in the chloroplast genome. Our Northern blot analysis indicated that *psb*L and *psb*J are co-transcribed as a polycistron and are not subjected to further processing into smaller transcripts. We also determined that varying daylight intensities (5, 10, 20, or 100%) did not significantly change the level of *in vivo* accumulation of *psb*LJ transcript.

Keywords: light intensity, *Panax ginseng*, Photosystem II, *psb*J, *psb*L, transcription

Photosystem II (PSII) is a major functional complex in the thylakoid membranes of chloroplast, in which light energy is converted into electrochemical energy. PSII consists of more than 10 different polypeptides. The primary photochemical reaction is performed by the PSII reaction center, which comprises D1, D2, and the cytochrome *b*-559 α- and β-subunits (Nanba and Sato, 1987). Cytochrome *b*-559 is present in the PSII reaction center from primitive oxygenic photosynthetic organisms to higher plants, implying that it has a structural role in maintaining functional integrity of the PSII reaction center (Pakrasi et al., 1988). It is generally accepted that cytochrome *b*-559 does not participate in the main electron transport pathway of the water oxidation by the PSII reaction center, but that its obligatory presence is related to the protection of PSII, which is labile and vulnerable to environmental stresses such as heat and high light intensity (Jang and Tae, 1996).

The *psb*E and *psb*F genes, encoding cytochrome *b*-559 α- and β-subunits, respectively, are co-transcribed as a polycistron. Their transcript size is larger than might be expected, as shown from Northern blot analysis. This implies that a polycistron contains another gene transcript(s) that is, therefore, co-transcribed with the *psb*EF genes. In higher plants, the *psb*L and *psb*J genes are transcribed with the *psb*EF gene (Haley and Bogorad, 1990). Although function of the *psb*L and *psb*J gene products in PSII has not been resolved, Kitamura et al. (1994) have suggested that the *psb*L protein, simultaneously reconstituted with both plastoquinone-9 (PQ-9) and thylakoid lipids in the PQ-9 depleted PSII reaction center core complex, could restore Q_A activity in that complex. However, such a recovery mechanism does not seem to be a result from PQ-9 stabilization in the Q_A site of PSII. In fact, the carboxy terminal domain, rather than the amino terminal domain, of the *psb*L protein was revealed to be crucial for recovering electron transfer activity (Ozawa et al., 1997).

P. ginseng C. A. Meyer, a perennial herb in the family *Araliaceae*, is cultivated for medicinal purposes in Korea. Because ginseng is a shade-grown plant, light intensity may be a limiting factor among the environmental stresses that affect its growth rate. The most extensive studies have focused on the effect of high light intensities on photosynthesis, especially the light reaction. The photosynthetic activity decreases (Cheon, 1989) and the composition of chlorophyll-protein complexes and integral proteins in thylakoids are changed when light intensity is >2000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (Degreef et al., 1971; Bushmann et al., 1978). Moreover, chlorophyll content declines while the chlorophyll *a*/*b* ratio increases when ginseng leaves are exposed to high light. Nevertheless, only a few studies have concentrated on possible structural and functional effects of high light intensity on the essential proteins of PSII in shade-grown species.

To better understand the functional role(s) of *psb*L

*Corresponding author; fax +82-41-551-9229
e-mail gtae@dankook.ac.kr

and *psb*J gene products in the PSII complex, we cloned those two genes from the chloroplast genome of *P. ginseng* and analyzed their primary structures and deduced amino acid sequences. In addition, we investigated the effects of various intensities of daylight on transcriptional activity of the *psb*LJ gene.

MATERIALS AND METHODS

Plant Materials and Isolation of Chloroplast Genomic DNA

P. ginseng plants were grown in the field under a 12-h photoperiod and various intensities of daylight (5, 10, 20, or 100%). Fully expanded, mature leaves were collected, frozen in liquid nitrogen, and stored at -70°C. The frozen tissues were then ground in a mortar with liquid nitrogen. For each 10-g sample, the powder was suspended in 100 mL of extraction buffer (50 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.35 M sorbitol, 0.1% BSA, 0.1% β-mercaptoethanol, and 10 % PEG 4000). The homogenate was filtered through several layers of cheesecloth and one layer of miracloth. Chloroplasts were pelleted by centrifugation at 8000*g* for 15 min and resuspended in 5 mL of washing buffer (10 mM Tris-HCl pH 8.0, 20 mM EDTA, 0.35 M sorbitol, and 0.1% β-mercaptoethanol). Afterward, 1 mL of 5% sarkosyl was added. This mixture was then incubated for 15 min at room temperature (RT) before 860 μl of 5 M NaCl and 686 μl of 8.6% CTAB/0.7 M NaCl were added. The samples were incubated at 60°C for 15 min and extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). After centrifugation at 5000*g* for 10 min, the upper aqueous phase was collected and the nucleic acids were precipitated by adding 2/3 volume of isopropanol. Following incubation for 10 min at RT, the nucleic acids were pelleted by centrifugation at 14300*g* for 20 min. The pellets were then washed with 70% ice-cold ethanol, air-dried, and re-suspended in an appropriate volume of water or TE buffer.

Cloning and Sequence Analysis of *psb*LJ Genes

A polymerase chain reaction (PCR) was performed with chloroplast genomic DNA serving as template. Two different primers were used: forward, 5'-AAATTT TCGSRATCAATGRTTGGACYATGC-3'; R=G/A, S=G/C, Y=C/T; and reverse, 5'-AARAATWTKKGGAGYTCR GC-3'; K=G/T, W=A/T. These forward and reverse primers were designed to bind to the upstream regions of the *pet*A and the *psb*E genes, respectively. PCR included 35 cycles of 1 min at 95°C for denaturation, 1.5 min at 47°C for annealing, and 2 min at 72°C for chain elongation. The reaction medium contained 0.2 mM dNTP, 1 μM of forward primer, 1 μM of reverse primer, and 2 units *Taq* DNA polymerase (Promega, USA). PCR products were analyzed on a 0.8% agarose gel. Approximately 2.9 kb of DNA was extracted with a Geneclean kit (BIO 101, CA, USA) and ligated to the pGEM-T Easy vector (Promega) with T4 DNA ligase (Promega) in the presence of 10 mM DTT, 30 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, and 1 mM ATP. The ligation mixture was then transformed into *Escherichia coli* strain JM109. A plasmid containing the insert was extracted from a white colony grown in the presence of X-gal, and the insert size was analyzed via restriction digestion mapping.

DNA sequencing of the *psb*LJ genes in a pGEM-T Easy vector was performed with an automated DNA sequence analyzer (LI-COR Biotechnology, Model Long Read IR 4200). The resulting sequence data were then compared by blast search with the National Center for Biotechnology Information (NCBI) database to estimate the degree of identity with *psb*L and *psb*J genes from dicots, monocots, and a liverwort. Alignment of the amino acid sequences was performed with the shareware program, SeqPup.

Preparation and Labeling of *psb*LJ Gene-specific Probe

To prepare the *psb*LJ gene-specific DNA probe, we designed two primers to bind upstream of *psb*L (5'-GAGCTATGACACAATCAAAC-3') and downstream of *psb*J (5'-GATTACTACAGGGATGAACC-3'). These primers were used to amplify the DNA fragment containing the *psb*L and *psb*J genes. A *psb*EF gene-specific DNA probe was prepared and labeled according to Lee et al. (1998). The DNA sequence containing the *psb*EFLJ genes was amplified with two different primers: forward, 5'-AARAATWTKKGGAGYTCRGC-3'; R =G/A, W=A/T, K=G/T, Y=C/T; and reverse, 5'-GATTACTACAGGGATGAACC-3'. The PCR product was subcloned into a pGEM-T Easy vector, and the insert was cleaved with *Eco*RI and then DIG-labeled (Boehringer Mannheim GmbH, Mannheim, Germany). After 1 μg of the insert was boiled for 10 min and placed on ice, we added 20 μl of the reaction mixture containing 50 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 0.1 mM dithioerythreitol, 0.2 mg/mL BSA, 0.1 mM dATP, 0.1 mM dCTP, 0.065 mM dTTP, 0.035 mM alkali-labile DIG-dUTP (pH 6.5), and 2 units of a Kle-

now fragment. This mixture was incubated for 20 h at 37°C. The DIG-labeled probe was then mixed with hybridization fluid containing 50% (v/v) deionized formaldehyde, 5X SSC (150 mM NaCl and 15 mM sodium citrate), 0.1% (w/v) sodium lauryl sarcosine, 0.02% SDS, and 2% (w/v) blocking reagent (Boehringer Mannheim) and stored at -20°C.

Northern and Southern Blot Analyses

Total RNA was extracted from the leaf tissue with an RNeasy mini kit (Qiagen, USA). The integrity of mRNA was indirectly measured based on the intactness of rRNAs on a 1.0% agarose/formaldehyde denaturing gel (Sambrook et al., 1989). Non-radioactive Southern and Northern blot analyses were performed with DIG-labeled probes, according to the method of Lee et al. (1998). For the latter, approximately 10 mg of total RNA was separated on a 1.0% agarose/formaldehyde gel, transferred to a Magnagraph nylon membrane (MSI, USA) in 10X SSC (pH 7.0), and cross-linked with a UV light crosslinker (Hoefer Scientific, USA). The blot was then hybridized for 12 h at 42°C in a solution containing 5X SSC (pH 7.0), 50% (v/v) deionized formamide, 0.1% (w/v) sodium lauryl sarcosine, 0.02% (w/v) SDS, and 2% (w/v) blocking reagent. After hybridization, the blot was washed twice for 15 min at 42°C in 2X SSC containing 0.1% (w/v) SDS, and twice again for 15 min at 55°C in 0.5X SSC containing 0.1% (w/v) SDS. For our Southern blot analysis, approximately 10 μg of the purified chloroplast genomic DNA was digested with either *Bam*HI/*Hin*dIII or *Eco*RI/*Pst*I, electrophoresed on a 0.8% agarose gel in 1X TAE buffer, and transferred to a Magnagraph nylon membrane. Hybridization and washing procedures were carried out as with the Northern blot analysis.

RESULTS AND DISCUSSION

Nucleotides and Deduced Amino Acid Sequence Analysis of *psb*L and *psb*J Genes

We analyzed an approximately 2.9-kb DNA fragment from the *P. ginseng* chloroplast chromosome, and found at least two uninterrupted open reading frames (ORFs; data not shown). Comparisons of its nucleotide and deduced amino acid sequences with those from monocots and dicots revealed two ORFs with significant degrees of identities to *psb*L (117 nucleotides) and *psb*J (123 nucleotides) for PSII (Fig. 1). ATG initiation codons of the *psb*L and *psb*J genes are positioned at +1 and +250, respectively, and are connected throughout 132 nucleotides (+118 to +249), the area in which a potential ribosome-binding site is located. The tetranucleotide sequence, GGAG (+233 to +236), is complimentary to that from the 3′ end of 16S rRNA in both tobacco (Tohdoh and Sugiura, 1982) and *E. coli* (Shine and Dalgarno, 1974). More than 90% of the chloroplast genes encoding proteins in higher plants possess an upstream sequence similar to the bacterial Shine-Dalgarno (SD) sequence. However, spacing of these chloroplastic SD-like sequences is less conserved, ranging from -2 to -29 (Ruf and Kossel, 1988; Bonham-Smith and Bourque, 1989; Gillham et al., 1994). Furthermore, about 6% of the chloroplast mRNAs in higher plants have no SD-like sequence at all (Bonham-Smith and Bourque, 1989). Whether this ribosome-binding sequence, located 12 nucleotides upstream from the initiation codon of the *psb*J gene, plays a functional role in translation remains to be demonstrated.

The initiation codon of the *psb*L gene in tobacco (Chaudhuri and Maliga, 1996) and spinach (Bock et al., 1993) is ACG, which is altered to AUG post-transcriptionally. This process, called RNA editing, has been detected in divergent organisms, from mammals

```
1 ——►  psbL gene                                                    45
ATG ACA CAA TCA AAC CCG AAC GAA CAA AAT GTT GAA TTG AAT CGT
Met Thr Gln Ser Asn Pro Asn Glu Gln Asn Val Glu Leu Asn Arg

46                                                                  90
ACC AGC CTC TAC TGG GGG TTA TTA CTC ATT TTT GTA CTT GCT GTT
Thr Ser Leu Tyr Trp Gly Leu Leu Leu Ile Phe Val Leu Ala Val

91                                                                 137
TTA TTT TCC AAT TAT TTC TTC AAT TAA    GAAAACGAAGGAGAATAATA
Leu Phe Ser Asn Tyr Phe Phe Asn  *

138                                                                193
ATTCTAGGCACTCTCTCTTAGCCCATTCGGAAGGATCTCATCTCATAATTATCCAT

194                                                                249
GACTGTTTGTGTCTCTAGCACGACCGCTTGATGAAATGTGGAGGGAAGTGGGGTAA

250 ——►  psbJ gene                                                 295
ATG GCT GAT ACT ACT GGA AGG ATT CCT CTT TGG ATA ATA GGT ACT
Met Ala Asp Thr Thr Gly Arg Ile Phe Leu Trp Ile Ile Gly Thr

296                                                                340
GTA GCT GGT ATT CTT GTG ATT GGT TTA ATA GGT ATT TTT TTT TAT
Val Ala Gly Ile Leu Val Ile Gly Leu Ile Gly Ile Phe Phe Tyr

341                                          373
GGT TCA TAT TCC GGA TTG GGT TCA TCC CTG TAG
Gly Ser Tyr Ser Gly Leu Gly Ser Ser Leu  *
```

Figure 1. Nucleotide and deduced amino acid sequences of the *psb*L and *psb*J genes. Amino acid sequences contain full-length gene products. A total of 419 nucleotides are presented and numbered in 5′ to 3′ direction; the first nucleotide of *psb*L is designated as +1. Potential ribosome-binding site is boxed, and hydrophobic domains of gene products are underlined.

to viruses to higher plants (Chan, 1993; Innerarity et al., 1996). The translational initiation codon of the *psb*L gene is only one of approximately 25 editing sites found in the plastids of higher plants (Maier et al., 1995). In contrast, the mitochondria are estimated to have >1000 sites (Schuster and Brennicke, 1994). In lower plants, RNA editing seems to occur more extensively in both the chloroplasts and the mitochondria, and involves the change of C to U as well as U to C. Despite this editing event being so widespread in plants, its significance is still unclear.

The deduced amino acid sequence of our *psb*L gene shows >97% identity to higher plants such as dicots and monocots, but <80% identity to lower organisms, e.g., *Chlamydomonas reinhardtii* and *Synechocystis* sp. PCC 6803 (Fig. 2A). For the *psb*J gene, the identity to higher plants is more than 93% and to lower organisms is ranging from 48 to 90% (Fig. 2B). Therefore, the *psb*L gene seems to be more conserved than the *psb*J gene, the latter also showing larger variations in identity among lower organisms. These results imply that the *psb*L gene product is more responsible for the photosynthetic activity of PSII.

Genomic Southern Blot Analysis of *psb*LJ Genes

DNA fragments of the *psb*LJ and *psb*EF genes were DIG-labeled and used as probes to determine the copy number for *psb*LJ in the chloroplast genome under high stringency conditions. Approximately 10 mg of purified chloroplast DNA was used for our Southern blot analysis, after being digested with restriction enzymes *Bam*HI/*Hin*dIII (Fig. 3, Lane 2) and *Eco*RI/*Pst*I (Fig. 3, Lane 3). When the blot was probed with the *psb*LJ genes (Fig. 3A), a single band was observed in each of Lanes 2 and 3, with sizes of approximately 3.50 kb and 1.45 kb, respectively. A similar pattern was obtained when probing was done with the *psb*EF genes (Fig. 3B), except that two bands were observed in Lane 3. This result implies that *psb*EF lacks *Eco*RI or *Pst*I sites. Extensive DNA sequence analysis and restriction mapping indicated a single site of *Eco*RI present in the *psb*E gene (Fig. 4, upper panel).

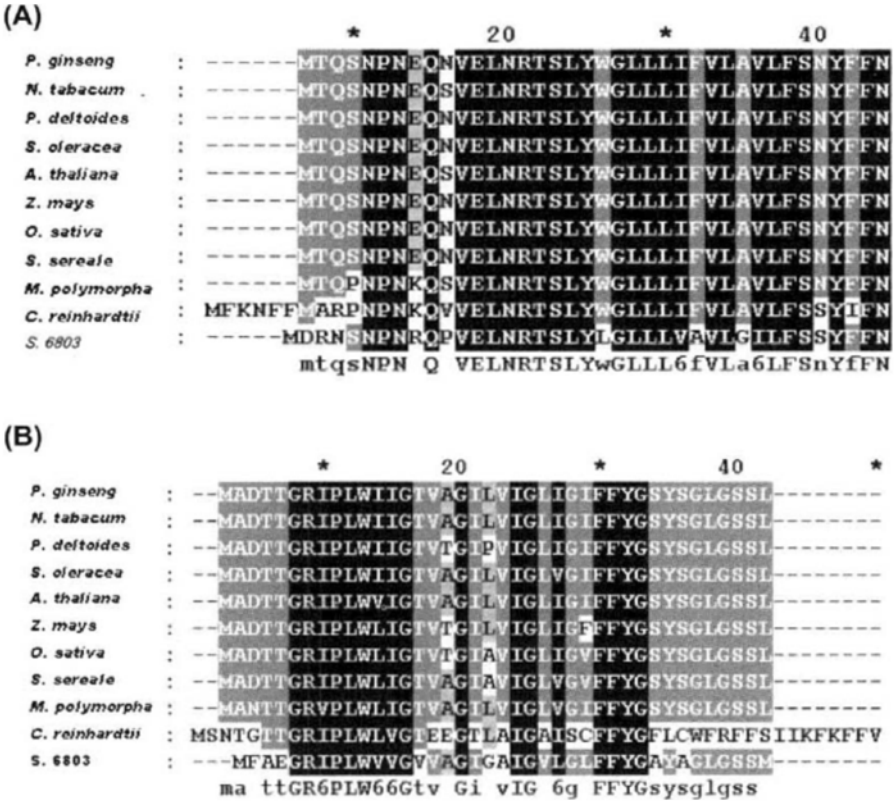

Figure 2. Comparisons of amino acid sequences for gene products of *psb*L (**A**) and *psb*J (**B**) from *P. ginseng* with those from dicots [*Nicotiana tabacum* (Z00044), *Populus deltoides* (X89651), *Spinacia oleracea* (AJ400848), *Arabidopsis thaliana* (AP000423)]; monocots [*Zea mays* (X86563), *Oryza sativa* (X15901), *Secale cereale* (X13326)]; and other organisms [*Marchantia polymorpha* (NC_001319), *Chlamydomonas reinhardtii* (BK000554), *Synechocystis* sp. PCC 6803 (NC_000911)]. Multiple sequence alignments were performed with GENEDOC and CLUSTALW programs.

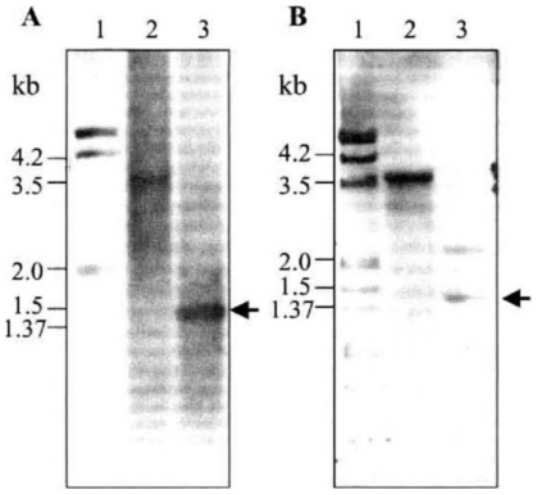

Figure 3. Genomic Southern blot analysis of *P. ginseng* chloroplast *psb*LJ and *psb*EF genes. Genomic DNA was digested with *Bam*HI and *Hin*dIII (Lane 2) or *Eco*RI and *Pst*I (Lane 3), then separated on a 0.8% agarose gel and transferred to a Magnagraph nylon membrane. Blots were hybridized with DIG-labeled *psb*LJ (**A**) or *psb*EF (**B**) gene-specific probes. Lane 1 is DIG-labeled DNA marker.

Band sizes were approximately 2.20 kb and 1.45 kb, the latter being the same size as that seen in Figure 3A, Lane 3. Because both probes, specific for the *psb*LJ and *psb*EF genes, could detect bands of 3.50 kb and 1.45 kb in Lanes 2 and 3, respectively, we believe the *psb*LJ genes are likely to be clustered with the *psb*EF genes. In addition, the *psb*LJ genes appear as single copies in the *P. ginseng* chloroplast genome, based on our Northern blot analysis, thereby demonstrating that they are co-transcribed with the *psb*EF genes.

Northern Blot Analysis of *psb*LJ Genes

We evaluated the message of the *psb*LJ genes, using total RNA extracted from leaves of *P. ginseng* grown under different light intensities. Analysis of the relevant species of 16S, 18S, and 25S rRNA on the agarose/formaldehyde gels indicated that the mRNA was relatively intact and that equal amounts had been loaded. To detect the transcript of *psb*LJ, its PCR-amplified and DIG-labeled DNA fragment was used as Probe A. A single band was detected with probe A at the position between the 16S and 18S rRNA bands, the size of which was approximately 1.5 kb (Fig. 4A). Another DIG-labeled probe for the *psb*EF genes (Probe B) also was able to recognize that band, thereby implying that the *psb*LJ genes are co-transcribed with the *psb*EF genes (Fig. 4B). A third DIG-labeled probe

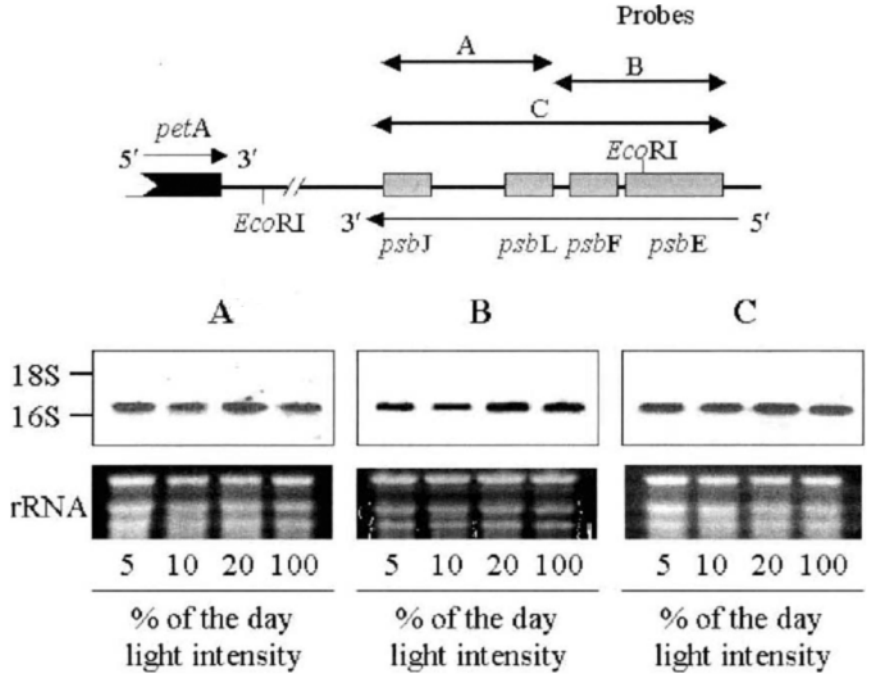

Figure 4. Northern blot analysis of chloroplast *psb*EFLJ genes from *P. ginseng*. Total RNA was isolated from leaves of plants grown under different intensities of daylight (5, 10, 20, or 100%). Approximately 10 µg of purified RNA was separated on a 1.0% agarose/formaldehyde denaturing gel and transferred to nylon membrane. DIG-labeled *psb*LJ (**A**), *psb*EF (**B**), and *psb*EFLJ (**C**) gene-specific probes were used to measure *in vivo* accumulation of mRNA. Ethidium bromide-stained rRNAs bands were compared to ensure that equal amounts of RNA were loaded onto each lane.

(Probe C) that encompassed the *psb*EFLJ genes was prepared and analyzed via Northern blot analysis. This probe was hybridized to the *psb*LJ mRNA as a single band (Fig. 4C), indicating that the *psb*LJ genes constitute a polycistron with the *psb*EF genes, and that no intercistronic cleavage occurs in the *P. ginseng* chloroplast.

Because *P. ginseng* is a shaded-grown crop, high light intensity causes photoinhibition of PSII. Therefore, we investigated the possible involvement of the *psb*LJ genes in this phenomenon. After plants were cultivated under 5, 10, 20, or 100% daylight, we measured *in vivo* accumulation of *psb*LJ transcripts, using Northern hybridization techniques and Probes A, B, and C. This analysis revealed no significant change in the steady-state level of *psb*LJ mRNA as a result of varying light intensities (Fig. 4A-C).

In conclusion, we have demonstrated here that *psb*LJ genes from *P. ginseng*, comprising 117 or 123 nucleotides, are co-transcribed with the *psb*EF genes as a polycistron, and that transcription rates of the former are apparently not significantly affected by fluctuating light intensities. Therefore, we believe that the transcriptional level of *psb*LJ gene expression is not responsible for photoinhibition in that species.

ACKNOWLEDGEMENT

We thank Won-Kyu Lee for his advice and technical assistance.

Received March 19, 2004; accepted June 2, 2004.

LITERATURE CITED

Bock R, Hagemann R, Kossel H, Kudla J (1993) Tissue and stage specific modulation of RNA editing of the *psb*F and *psb*L transcript from spinach plastids – a new regulatory mechanism? Mol Gen Genet 240: 238-244

Bonham-Smith PC, Bourque DP (1989) Translation of chloroplast-encoded mRNA: Potential initiation and termination signals. Nucleic Acids Res 19: 155-162

Bushmann C, Melier D, Klendgen HK, Lichtenthaler HK (1978) Regulation of chloroplast development by red and blue light. Photochem Photobiol 27: 195-198

Chan L (1993) RNA editing: Exploring one mode with apolipoprotein B mRNA. BioEssays 15: 33-41

Chaudhuri S, Maliga P (1996) Sequences directing C to U editing of the plastid *psb*L mRNA are located within a 22 nucleotide segment spanning the editing site. EMBO J 15: 5958-5964

Cheon SK (1989) Effects of light intensity and quality on the growth and quality of Korean ginseng. Ph. D. thesis. Kyungbook National University, Daegu

Degreef J, Butler WL, Roth TF (1971) Greening of etiolated bean leaves by far-red light. Plant Physiol 47: 457-464

Gillham NW, Boynton JE, Hauser CR (1994) Translation regulation of gene expression in chloroplasts and mitochondria. Annu Rev Genet 28: 71-93

Haley J, Bogorad L (1990) Alternative promoters are used for genes within maize chloroplast transcription units. Plant Cell 2: 323-333

Innerarity TL, Boren J, Yamanaka S, Olofsson SO (1996) Biosynthesis of apolipoprotein B48-containing lipoproteins. J Biol Chem 271: 2353-2356

Jang WC, Tae GS (1996) Structural changes of the spinach photosystem II reaction center after inactivation by heat treatment. J Biochem Mol Biol 29: 58-62

Kitamura K, Ozawa S, Shiina T, Toyoshima Y (1994) L protein, encoded by *psb*L, restores normal functioning of the primary quinone acceptor, Q-A, in isolated D1-D2-CP47-Cytb-559-I photosystem II reaction center core complex. FEBS Lett 354: 113-116

Lee WK, Park DS, Tae GS (1998) Cloning and characterization of the *psb*EF gene encoding cytochrome b-559 of the *Panax ginseng* photosystem II reaction center. J Biochem Mol Biol 32: 189-195

Maier RM, Neckermann K, Igloi GL, Kössel H (1995) Complete sequence of the maize chloroplast genome: Gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. J Mol Biol 251: 614-628

Nanba O, Satoh K (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome *b*-559. Proc Natl Acad Sci USA 84: 109-112

Ozawa S, Kobayashi T, Sugiyama R, Hoshida H, Shiina T, Toyoshima Y (1997) Role of PSII-L protein (*psb*L gene product) on the electron transfer in photosystem II complex: 1. Over-production of wild-type and mutant versions of PSII-L protein and reconstitution into the PSII core complex. Plant Mol Biol 34: 151-161

Pakrasi HB, Williams JGK, Arntzen CJ (1988) Targeted mutagenesis of the *psb*E and *psb*F genes blocks photosynthetic electron transport; Evidence for a functional role of cytochrome *b*-559 in Photosystem II. EMBO J 7: 325-332

Ruf M, Kossel H (1988) Occurrence and spacing of ribosome recognition sites in mRNA of chloroplasts from higher plants. FEBS Lett 240: 42-44

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York

Schuster W, Brennicke A (1994) The plant mitochondrial genome: Physical structure, information content, RNA editing, and gene migration. Annu Rev Plant Physiol Plant Mol Biol 45: 61-78

Shine J, Dalgarno L (1974) The 3'-terminal sequence of *Escherichia coli* 16S rRNA complementarity to non-

sense triplets and ribosome binding sites. Proc Natl Acad Sci USA 71: 1342-1346

Tohdoh N, Sugiura M (1982) The complete nucleotide sequence of 16S ribosomal RNA gene from tobacco chloroplasts. Gene 17: 213-218