Molecular Cloning and Characterization of the psbL and psbJ Genes for Photosystem II from Panax ginseng

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We have cloned and characterized two genes for Photosystem II from chloroplasts of Panax ginseng. These genes, psbL and psbJ, 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 psbL and psbJ 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 psbL transcript.

Keywords: light intensity, Panax ginseng, Photosystem II, psbJ, psbL, 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 psbE and psbF 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 psbEF genes. In higher plants, the psbL and psbJ genes are transcribed with the psbEF gene (Haley and Bogorad, 1990). Although function of the psbL and psbJ gene products in PSII has not been resolved, Kitamura et al. (1994) have suggested that the psbL 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 psbL 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 μmol-m$^{-2}$-sec$^{-1}$ (Degwe 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 psbL...
and psbJ 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 psbLJ 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 8000g 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 5000g 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 14300g 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 psbLJ Genes**

A polymerase chain reaction (PCR) was performed with chloroplast genomic DNA serving as template. Two different primers were used: forward, 5'-AAATTT TCGGATCTATCCGACATGCCGCGC-3'; R = G/A, S = G/C, Y = C/T; and reverse, 5'-AAATTTTGGGTCGTCCGAGGTCG CGC-3'; K = G/T, W = A/T. These forward and reverse primers were designed to bind to the upstream regions of the petA and the psbE 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 Gene-clean 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 MgCl₂, 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 psbLJ 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 psbL and psbJ genes from dicots, monocots, and a liverwort. Alignment of the amino acid sequences was performed with the shareware program, SeqPup.

**Preparation and Labeling of psbLJ Gene-specific Probe**

To prepare the psbLJ gene-specific DNA probe, we designed two primers to bind upstream of psbL (5'-GAGCTATAGACATAACAC-3') and downstream of psbJ (5'-AAGTTAACGTGGGATGAC-3'). These primers were used to amplify the DNA fragment containing the psbL and psbJ genes. A psbEFLJ gene-specific DNA probe was prepared and labeled according to Lee et al. (1998). The DNA sequence containing the psbEFLJ genes was amplified with two different primers: forward, 5'-AAAGGATATCTTTGCGTAC-3'; R = G/A, W = A/T, K = G/T, Y = C/T; and reverse, 5'-GATGTAAGGATGGATGAC-3'. The PCR product was subcloned into a pGEM-T Easy vector, and the insert was cleaved with EcoRI 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 MgCl₂, 0.1 mM dithioerythritol, 0.2 mg/ml BSA, 0.1 mM dUTP, 0.1 mM dCTP, 0.065 mM dGTP, 0.035 mM alkali-labile DIG-dUTP (pH 6.5), and 2 units of a Kle-