Journal of Photoscience (2003), Vol. 10(3), pp. 245-249

Cloning and characterization of the *psb*A Gene from *Panax ginseng* (Characterization of the *psb*A Gene from *P. ginseng*)

Won-Kyu Lee and Gun-Sik Tae*
Department of Biology, College of the Advanced Science,
Dankook University, Cheoan 330-714 Korea

The *psb*A gene of photosystem II was cloned and characterized from the *P. ginseng* chloroplast. The *psb*A gene is composed of 1,062 nucleotides. The overall amino acid sequence shows 99% and 98% identities to dicots and monocots of higher plants, respectively. Southern blot analysis revealed that a single copy of the *psb*A gene existed in the chloroplast genome. Northern blot analysis of the *in vivo* accumulation of the *psb*A transcript, after being grown under the different intensities (5%, 10%, 20%, and 100%) of daylight, indicated that the steady-state level of the *psb*A transcript was not significantly affected by light intensity.

key words: *psb*A, photosystem II, *Panax ginseng*, transcription, light intensity

INTRODUCTION

Photosytem II (PSII) is one of the major functional complexes of the thylakoid membranes in chloroplast, in which the light energy is converted into the electrochemical energy. PSII consists of more than 25 different polypeptides, which contain a number of cofactors that mediate the charge separation across the membrane. The PSII reaction center, consisting of D1, D2, and the cytochrome *b-559* α- and β-subunits, was isolated from spinach chloroplast [1, 2] and the primary charge separation in PSII was located on a heterodimer consisting of the D1 and D2 polypeptides, encoded by the *psb*A and *psb*D genes, respectively.

The D1 and D2 polypeptides are homologous proteins, interacting strongly with each other and sharing 1-2 reaction center chlorophyll *a* molecule (P680) and contain all the redox components participating in PSII electron transport from the primary donor P680 to the primary and the secondary quinone acceptors, Q_A and Q_B. High intensity of light causes mismatches in electron transport, which in turn could create oxidative damages on PSII, especially the D1 polypeptide. This phenomenon is known as photoinhibition. The damaged polypeptides are subsequently degraded [3, 4]. The D1 polypeptide is known to have a rapid turnover even in moderate light [5] and the turnover rate increases with increasing light intensity[6]. As a consequence, the D1 polypeptide needs to be replaced by a newly synthesized D1 polypeptide in order to restore the functional capability of the PSII reaction center [3, 7-9].

Chloroplast gene expression is strongly regulated by light in higher plants as well as other unicellular organisms such as *Chlamydomonas reinhardtii* and cyanobacteria. Light can modulate the overall plastid transcriptional activity and differentially stimulate the transcription of the PSII genes including the *psb*A gene, encoding the D1 polypeptide [10]. Therefore, the maintenance of the D1 polypeptide in PSII under various intensities of daylight requires the transcriptional and translational regulations of the *psb*A gene [11-14].

Panax ginseng C. A. Meyer, a perennial herb in the family of *Araliaceae*, is a shaded plant so that high light intensity could be a limiting factor that affects the growth rate. The most extensive studies were performed with regard to the effect of high light intensity on the light reaction. The photosynthetic activity decreases [15] and compositions of chlorophyll-protein complexes are changed if the light intensity is higher than 2,000 μEinstein/m^2 · sec [16]. Only a few studies have been, however, reported in the shaded plants about the effects of high light intensity on the transcriptional regulations of the essential proteins of the PSII reaction center.

In the present study, the *psb*A gene of the PSII reaction center was cloned from the chloroplast genome of *P. ginseng* and its primary structures and deduced amino acid sequences were analyzed. In addition, the effects of various intensities of daylight on transcriptional activity of the *psb*A gene were investigated. This would be helpful in part to understand the protection mechanism of the PSII from photoinhibition.

MATERIALS AND METHODS

Plant Material and Isolation of Chloroplast Genomic DNA

P. ginseng was grown at the field under various intensities (5%, 10%, 20% and 100%) of daylight (approx. 1000 μ Einstein/m^2 · sec) with a 12hr-photoperiod. The mature leaves were collected,

*To whom correspondence should be addressed.
E-mail : gtae@dankook.ac.kr
Received November 28, 2003; Accepted December 11, 2003

frozen in liquid nitrogen, and stored at -70°C for further preparations. The frozen leaves were ground in a mortar with liquid nitrogen and the powder was suspended in 100 mL of the extraction buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 0.35M sorbitol, 0.1% BSA, 0.1% β-mercaptoethanol, 10% PEG 4000) for every 10 g of leaves. The homogenate was filtered through several layers of cheesecloth and one layer of miracloth. Chloroplasts were pelleted by centrifugation at 8,000*g* for 15 min and resuspended in 5 mL of the washing buffer (10 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.35M sorbitol, 0.1% β-mercaptoethanol). One milliliter of 5% sarkosyl was added and the mix was incubated for 15 min at room temperature. Then, 860 μl of 5M NaCl and 686 μl of 8.6% CTAB/0.7M 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 5,000*g* for 10 min, the upper aqueous phase was collected and nucleic acids were precipitated by adding 2/3 volume of isopropanol. After incubation for 10 min at room temperature, nucleic acids were pelleted by centrifugation at 14,300 *g* for 20 min. The pellet was washed with 70% ice-cold ethanol, air-dried, and resuspended in an appropriate volume of water or TE buffer.

Cloning and Sequence Analysis of the psbA Gene

A polymerase chain reaction (PCR) was performed with the chloroplast genomic DNA as a template. The two different primers were used as follows: the forward primer (5'-CGACTAGTTCCGGGTTCG-3') and the reverse primer (5'-GGCGAACGACGGGAATTG-3'). Thirty-five cycles of PCR (each of 1 min at 95°C for denaturation followed by 1.5 min at 55 °C for annealing and 2 min at 72°C for the chain elongation) were performed in a reaction medium including 0.2 mM dNTP, 1 μM the forward primer, 1μM the reverse primer, and 2 units of *Taq* DNA polymerase (Promega, Madison, USA). The PCR products were analyzed on 0.8% agarose gel. The DNA of approximately 1.8 kb in size was extracted with a Geneclean kit (BIO 101, CA, USA) and ligated to pGEM-T Easy vector (Promega, Madison, USA) with T4 DNA ligase (Promega, Madison, USA) 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 with an insert was extracted from a white colony grown in the presence of X-gal and the size of an insert was analyzed by the restriction digestion mapping. The DNA sequencing of the *psb*A gene in pGEM-T Easy vector was performed with the automated DNA sequence analyzer (LI-COR Biotechnology, Model long read IR 4200) and the DNA sequence data obtained were blasted to National Center for Biotechnology Information (NCBI) to estimate the degree of identity to the *psb*A gene from dicots, monocots, or a liverwort. The amino acid sequence alignment was performed with the shareware program, SeqPup.

Preparation and Labeling of the psbA Gene-Specific Probe

To prepare the *psb*A gene-specific DNA probe, DNA fragment of the *psb*A gene was amplified with two different primers: the forward primer 5'-CCTGTTTCAGGGTCTCTAC-3', and the reverse primer 5'-GGATGTTGTGTCAGCCTGG-3'. The PCR product was cloned into pGEM-T Easy vector. The insert was cleaved with *Eco*RI and DIG-labeled (Boehringer Mannheim GmbH, Mannheim, Germany). One microgram of an insert was boiled for 10 min and placed on ice. Twenty microliters of the reaction mixture containing 50 mM Tris-HCl, pH 7.2, 10 mM $MgCl_2$, 0.1 mM dithio-erythreitol, 0.2mg/mL BSA, 0.1 mM dATP, 0.1 mM dCTP, 0.065 mM dTTP, 0.035mM alkalilabile DIG-dUTP, pH 6.5, and 2 units of Klenow fragment were added and incubated for 20 hrs at 37°C. The DIG-labeled probe was then mixed with the hybridization fluid containing 50% (v/v) deionized form-aldehyde, 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 GmbH, Mannheim, Germany) and stored at -20°C.

Northern and Southern Blot Analyses

Total RNA was extracted from the leaves with an RNeasy mini kit (Qiagen, Valencia, USA). The integrity of mRNA was indirectly measured based on the intactness of rRNAs on 1.0% agarose/formaldehyde denaturing gel [17]. Non-radio-active Southern and Northern blot analyses were performed with DIG-labeled probes [18]. For Northern blot analysis, approximately 10 μg of total RNA was separated on 1.0% agarose/form-aldehyde gel, transferred to Magnagraph nylon membrane (MSI, Westborogh, USA) in 10x SSC, pH 7.0, and then cross-linked with UV crosslinker (Hoefer Scientific, San Francisco, USA). The blot was hybridized for 12 hrs 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 Southern blot analysis, approximately 10 μg of the purified chloroplast genomic DNA was digested with *Eco*RI, *Pst*I, and *Eco*RI/*Pst*I, electrophoresed on 0.8% agarose gel in 1x TAE buffer, and transferred to Magnagraph nylon membrane (MSI, Westborogh, USA). Hybridization and washing procedures were carried out as in Northern hybridization.

RESULTS AND DISCUSSION

Nucleotide and Its Deduced Amino Acid Sequence Analysis of the psbA Gene

Analysis of approximately 1.5kb DNA fragment from the *P. ginseng* chloroplast chromosome has disclosed at least one uninterrupted open reading frame (ORF). Comparisons of nucleotide and its deduced amino acid sequences with those from monocots and dicots in database have revealed that the ORF shows significant degree of identities to the *psb*A gene of PSII, which is composed of 1,062 nucleotides (Fig. 1). The

deduced amino acid sequence of the *psb*A gene showed 99% and 98% of identities to dicots and monocots of higher plants, respectively, and less than 95% to *C. reinhardtii*. The degree of identity of the D1 polypeptide decreases in cyanobacteria (data not shown), in which the carboxyterminal domain has the extra 7 amino acid residues that are absent in all land plant [19].

Genomic Southern Blot Analysis of the psbA Gene

The DNA fragment of the *psb*A gene was DIG-labeled and used as probe to determine a copy number in chloroplast genome under high stringency conditions. Approximately 10 μg of the chloroplast genomic DNA was used and Southern blot analysis of the chloroplast genomic DNA digested with two different restriction endonucleases, *Eco*RI and *Pst*I, was performed. A single digestion with *Eco*RI and *Pst*I resulted in one band of approximately 4.2 kb and larger than 10 kb in size, respectively (Fig. 2, lanes E and P). The double digestion of both *Eco*RI and *Pst*I (Fig. 2, lane E+P) generated a single and smaller hybridizing fragment of approximately 3.3 kb in size, implying that there is no additional physically unlinked *psb*A gene copies in the *P. ginseng* chloroplast genome. However, this result cannot excluded the possibility that there is another tightly arranged gene copy in a tandem array in 3.3 kb DNA fragment since the size of the *psb*A gene is turned out to be about 1 kb. Extensive database searches of the *psb*A gene in the chloroplast genome of higher plant indicate that the *P. ginseng* chloroplast genome contains a single copy of the gene for the D1 polypeptide.

```
  1 ATGACTGCAATTTTAGAGAGACGCGAAAGCGAAAGCCTATGGGGTCGCTTCTGTAACTGG  60
    M  T  A  I  L  E  R  R  E  S  E  S  L  W  G  R  F  C  N  W
 61 ATAACTAGCACTGAAAACCGTCTTTACATTGGATGGTTTGGTGTTTTGATGATCCCTACC 120
    I  T  S  T  E  N  R  L  Y  I  G  W  F  G  V  L  M  I  P  T
121 TTATTGACGGCAACTTCTGTATTTATTATTGCCTTCATTGCTGCTCCTCCAGTAGACATT 180
    L  L  T  A  T  S  V  F  I  I  A  F  I  A  A  P  P  V  D  I
181 GATGGTATTCGTGAACCTGTTTCAGGGTCTCTACTTTACGGAAACAATATTATTTCCGGT 240
    D  G  I  R  E  P  V  S  G  S  L  L  Y  G  N  N  I  I  S  G
241 GCCATTATTCCTACTTCTGCAGCTATAGGTTTACATTTTTACCCAATCTGGGAAGCGGCA 300
    A  I  I  P  T  S  A  A  I  G  L  H  F  Y  P  I  W  E  A  A
301 TCCGTTGATGAATGGTTATACAACGGTGGTCCTTATGAACTAATTGTTCTACACTTCTTA 360
    S  V  D  E  W  L  Y  N  G  G  P  Y  E  L  I  V  L  H  F  L
361 CTTGGTGTTGCCTGTTACATGGGTCGTGAGTGGGAGCTTAGTTTCCGTCTGGGTATGCGA 420
    L  G  V  A  C  Y  M  G  R  E  W  E  L  S  F  R  L  G  M  R
421 CCTTGGATTGCTGTTGCATATTCAGCTCCTGTTGCAGCTGCTGCTGCTGTTTTCTTGATC 480
    P  W  I  A  V  A  Y  S  A  P  V  A  A  A  A  A  V  F  L  I
481 TACCCAATTGGTCAAGGAAGTTTTTCAGATGGTATGCCTCTAGGAATCTCTGGTACTTTC 540
    Y  P  I  G  Q  G  S  F  S  D  G  M  P  L  G  I  S  G  T  F
541 AATTTCATGATTGTATTCCAGGCTGAGCACAACATCCTTATGCACCCATTTCACATGTTA 600
    N  F  M  I  V  F  Q  A  E  H  N  I  L  M  H  P  F  H  M  L
601 GGCGTAGCTGGTGTATTCGGCGGCTCCCTATTCAGTGCTATGCATGGTTCCTTGGTAACT 660
    G  V  A  G  V  F  G  G  S  L  F  S  A  M  H  G  S  L  V  T
661 TCTAGTTTGATCAGGGAAACCACAGAAAATGAATCTGCTAATGAAGGTTACAGATTTGGG 720
    S  S  L  I  R  E  T  T  E  N  E  S  A  N  E  G  Y  R  F  G
721 CAAGAAGAAGAAACTTATAATATCGTAGCCGCTCATGGTTATTTTGGTCGATGGATCTTC 780
    Q  E  E  E  T  Y  N  I  V  A  A  H  G  Y  F  G  R  W  I  F
781 CAATATGCTAGTTTCAACAACTCTCGTTCTTTACATTTCTTCCTAGCTGCTTGGCCTGTA 840
    Q  Y  A  S  F  N  N  S  R  S  L  H  F  F  L  A  A  W  P  V
841 GTAGGTATCTGGTTCACTGCTTTAGGTATTAGCACTATGGCTTTCAACCTAAATGGTTTC 900
    V  G  I  W  F  T  A  L  G  I  S  T  M  A  F  N  L  N  G  F
901 AATTTCAACCAATCTGTAGTTGATAGTCAAGGCCGTGTAATTAATACTTGGGCTGATATC 960
    N  F  N  Q  S  V  V  D  S  Q  G  R  V  I  N  T  W  A  D  I
961 ATTAACCGTGCTAACCTTGGTATGGAAGTTATGCATGAACGAAATGCTCACAACTTCCCT 1020
    I  N  R  A  N  L  G  M  E  V  M  H  E  R  N  A  H  N  F  P
1021 CTAGACCTAGCTGCTATCGAAGCTCCATCTACAAATGGATAA                  1062
    L  D  L  A  A  I  E  A  P  S  T  N  G  *
```

Figure 1. Nucleotide and its deduced amino acid sequences of the *psb*A gene. The total 1,062 nucleotide sequence of the *psb*A gene of *P. ginseng* is presented and numbered in 5to 3direction. Its deduced amino acid sequence is shown in one-letter code.

Northern Blot Analysis of the psbA Gene

The messages of the *psb*A gene was analyzed with the total

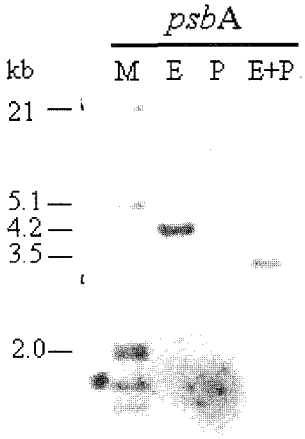

Figure 2. Genomic Southern blot analysis of the *P. ginseng* chloroplast *psb*A gene. Ten micrograms of the chloroplast genomic DNA were digested with the restriction endonucleases, *Eco*RI, *Pst*I, and both of *Eco*RI and *Pst*I in the lanes E, P, and E+P, respectively. The blot was hybridized with the DIG-labeled *psb*A gene-specific probe prepared with the random PCR technique.

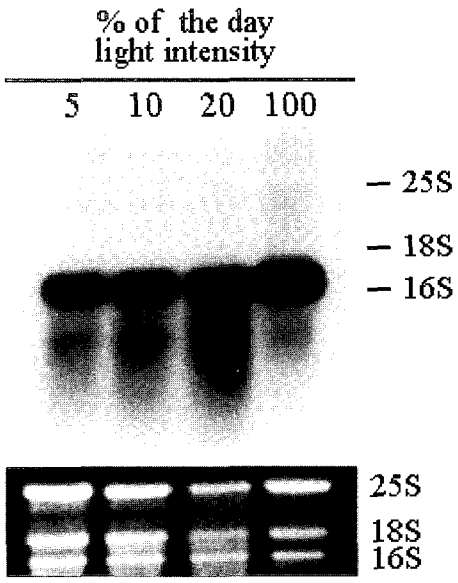

Figure 3. Effects of day light intensities on the *in vivo* accumulation of the *psb*A mRNA in the mature leaves of *P. ginseng*. Total RNA was extracted from mature leaves and analyzed in Northern blot analysis using a DIG-labeled *psb*A gene-specific probe. Ten micrograms of RNA were loaded in each lane.

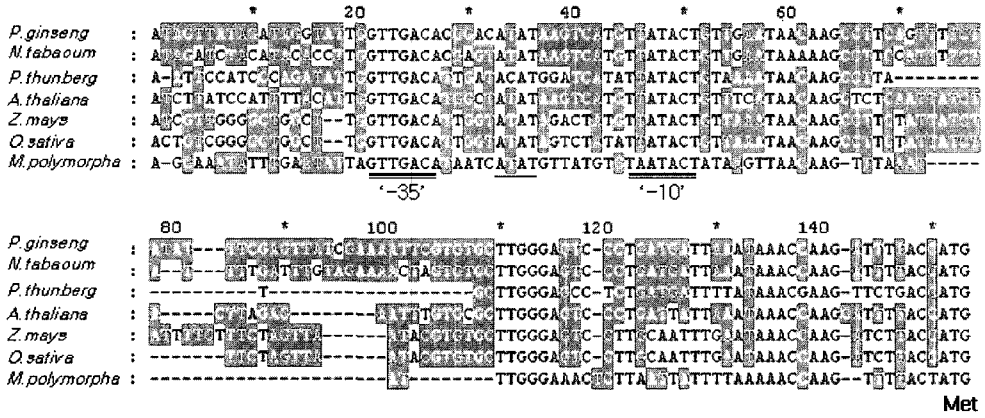

Figure 4. Nucleotide sequence comparison of the *P. ginseng psb*A promoter from those from six different plants. The nucleotide sequence of the *psb*A promoter region of *P. ginseng* is compared with those from dicots [*Nicotiana tabacum* (Z00044); *Pinus thunbergii* (D11467); *Arabidopsis thaliana* (AP000423)], monocots [*Zea mays* (X86563); *Oryza sativa* (X15901)], and other organisms [*Marchantia polymorpha* (NC_001319)]. Multiple sequence alignments were performed with the GENEDOC and the CLUSTALW softwares. The doubly underlined regions refer to the putative -35 and -10 elements.

RNA extracted from the leaves of *P. ginseng* grown under the different intensities (5%, 10%, 20% and 100%) of daylight. The intactness and the size distribution of the relevant species of 16S, 18S and 25S rRNA on agarose/formaldehyde gel indicated that mRNA was relatively intact (Fig. 3B). The equal amount of mRNA was loaded onto the gel for Northern blot analysis. In order to detect the transcript of the *psb*A gene, the PCR-amplified and DIG-labeled DNA fragment of the *psb*A gene was used as a probe. A single band was detected with the probe specific for the *psb*A gene at position near 16S rRNA band, of which the size was approximately 1.5 kb (Fig. 3A). Since *P. ginseng* is a shaded plant, high intensity of light causes the photoinhibition of PSII. The *psb*A gene product is known to be involved in protecting the PSII complex from photoinhibition by replacing the damaged D1 polypeptide very rapidly. The *in vivo* accumulation of the *psb*A transcript, after being cultivated under various intensities of daylight (5%, 10%, 20% and 100%), was measured and the results indicated that various intensities of daylight did not signifi-cantly affect the steady-state level of the *psb*A mRNA, implying that the transcriptional regulation of the *psb*A gene expression is not responsible for the protection of *P. ginseng* from photoinhibition. In the nucleotide sequence comparison of the *P. ginseng psb*A promoter from those of six different plants, the characteristic bacterial -10 and -35 consensus hexamer sequences, TATAAT and TTGACA, respectively, are found and the distance separating two consensus sequences is 18 bp, which is thought to be critical for RNA polymerase activity (Fig. 4).

Acknowledgements - The present research was conducted by the research fund of Dankook University in 2003.

REFERENCES

1. Nanba, O. and 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.
2. Ghanotakis, D. F., Demetriou, D. M. and Yocum, C. (1987) Isolation and characterization of an oxygen-evolving Photosystem II reaction center core preparation and a 28 kDa Chl-a-binding protein. *Biochem. Biophys. Acta* **891**, 15-21.
3. Prasil, O., Adir, N. and Ohad, I. (1992) Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. In J. Barber, ed., Topics in Photosynthesis, Vol. 11, pp.293-348, Elsevier, Amsterdam
4. Aro, E. M., McCaffery, S. and Anderson, J. (1993a) Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. *Plant Physiol.* **103**, 835-843.
5. Mattoo, A. K., Pick, U. Roffman-Falk, H. and Edelman, E. (1981) The rapidly metabolized 32,000 dalton polypeptide of the chloroplast is the "proteinaceous shield" regulating photosystem II electron transport and mediating diuron herbicide sensitivity. *Proc. Natl. Acad. Sci.* USA **78**, 1572-1576.
6. Kyle, D. J., Ohad, I. and Arntzen, C. J. C. J. (1984) Membrane protein damage and repair: selective loss of a quinone-proein function in chloroplast membranes. *Proc. Natl. Acad. Sci.* USA **81**, 4070-4074.
7. Adir, N., Shochat, S. and Ohad, I. (1990) Light-dependent D1 protein synthesis and translocation is regulated by reaction center II. *J. Biol. Chem.* **265**, 12563-12568.
8. Aro, E.-M., Virgin, I. and Andersson, B. (1993b) Photoinhibition of photosystem II. Inactivation, protein damage, and turnover. *Biochem. Biophys. Acta* **1143**, 113-134.
9. Ferjani, A., Abe, S. Ishikawa, Y. Henmi, T. Tomokawa, Y. Nishi, Y. Tamura, N. and Yamamoto, Y. (2001) Characterization of the stromal protease(s) degradation the cross-linked

products of the D1 protein generated by photoinhibition of photosystem II. *Biochem. Biophys. Acta* **1503**, 385-395.

10. Alfonso, M., Perewoska, I. and Kirilovsky, D. (2000) Redox control of *psb*A gene expression in the cyanobacterium *Synechocytis* PCC 6803. Involvement of the cytochrome b6/f complex. *Plant Physiol.* **122**, 505-515.
11. Rochaix, J.D. (1996) Post-transcriptional regulation of chloroplast gene expression in *Chlamydomonas reinhardtii*. *Plant Mol. Biol.* **32**, 327-341.
12. Bruick, R. K. and Mayfield, S. P. (1999) Light-activated translation of chloroplast mRNAs. *Trends Plant Sci.* **4**, 190-195.
13. Klein, R. R. and Mullet, J. E. (1990) Light-induced transcription of chloroplast genes *psb*A transcription is differentially enhanced in illuminated barley. *J. Biol. Chem.* **266**, 1895-1902.
14. Gamble, P. E. and Mullet, J. E. (1989) Translation *psb*B genes chloroplast and stability of proteins encoded by the plastid *psb*A and are regulated by a nuclear gene during light-induced development in barley. *J. Biol. Chem.* **264**, 7236-7243.
15. Cheon, S. K. (1989) Effects of light intensity and quality on the growth and quality of Korean ginseng. Ph. D. thesis, Kyungbook National University, Taegu, Korea
16. Degreef, J., Butler, W. L. and Roth, T. F. (1971) Greening of etiolated bean leaves by far-red light. *Plant Physiol.* **47**, 457-464.
17. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular cloning : A laboratory manual* (2nd edition). Cold Spring Harbor Laboratory. New York.
18. Lee, W-K., Park, D-S. and Tae, G-S. (1998) Cloning and characterization of the *psb*EF gene encoding cytochrome b-559 of the *Panax ginseng* potosystem II reaction center. *J. Biochem. Mol. Biol.* **32**, 189-195.
19. Hess, W. R., Weihe, A. Loiseaux-de Goer, S. Parensky, F. and Vaulot, D. (1995) Characterization of the single *psb*A gene of *Prochlorococcus marinus* CCMP 1375 (Prochloro-phyta). *Plant Mol. Biol.* 27, 1189-1196.