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The *psbE-F-L-J* operon from chloroplast genome of *Populus deltoides*: cloning, nucleotide sequence and transcript analysis

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Abstract. A 5·5-kbp *Hin*dIII fragment carrying *psbE-F-L-J* operon from the chloroplast DNA of *Populus deltoides* was cloned and nucleotide sequence was determined for a 1672-bp region. The coding regions showed more than 90% homology at nucleotide sequence level with plastid-encoded *psbE*, *psbF*, *psbL* and *psbJ* genes of higher plants. Pairwise alignments of *psbE*, *psbF*, *psbL* and *psbJ* coding regions of poplar with published sequences from other plants were carried out to analyse the nature of nucleotide substitutions. The 5′ and 3′ untranslated regions of the genes revealed high variability among different organisms both in terms of homology and the number of nucleotides. Northern hybridization data indicated that all four genes of *psbE-F-L-J* operon were transcribed as a single tetracistronic message which was not subjected to further processing into smaller transcripts. The transcript showed quantitative increase in mature leaves.

Keywords. Cyt b_{559}; *psbE*; *psbF*; *psbL*; *psbJ*; chloroplast genome; poplar.

1. Introduction

The photosystem II (PSII) reaction centre core of the chloroplast thylakoid membrane comprises D1 and D2 polypeptides and the α and β subunits of cytochrome b_{559} (Nanba and Satoh 1987). Both subunits of cyt b_{559} are oriented with the NH_2-terminus on the stromal side and the COOH-terminus on the lumenal surface of the photosynthetic membrane (Tae *et al.* 1988). Both the subunits are needed for stable formation of the PSII reaction centre (Pakrasi *et al.* 1991).

The genes for α and β subunits of cyt b_{559} have been mapped to a single locus in cyanobacteria and chloroplasts of green plants. The *psbE* gene encodes an 83-residue α subunit of cyt b_{559}. Immediately downstream and within the same operon are the *psbF* gene encoding the 39-amino-acid-long β-subunit polypeptide; *psbL* gene for L protein, possibly stabilizing Q_A, the first in the series of two quinone acceptors of PSII (Anbudurai and Pakrasi 1993); and *psbJ* gene for J protein, also associated with photosystem II (Pakrasi *et al.* 1991; Lind *et al.* 1993) The *psbE-F-L-J* operon from *Triticum aestivum* (Hird *et al.* 1986), *Oenothera hookeri* (Carillo *et al.* 1986), *Marchantia polymorpha* (Ohyama *et al.* 1986), *Nicotiana tabacum* (Shinozaki *et al.* 1986), *Cyanophora paradoxa* (Cantrell and Bryant 1988), *Euglena gracilis* (Cushman *et al.*

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1988a), *Synechocystis* 6803 (Pakrasi *et al.* 1988), *Secale cereale* (Kolosov *et al.* 1989), *Hordeum vulgare* (Krupinska and Berry-Lowe 1988), *Spinacia oleracea* (Westhoff *et al.* 1985), *Oryza sativa* (Hiratsuka *et al.* 1989), *Pinus thunbergii* (Wakasugi *et al.* 1994), *Zea mays* (Haley and Bogorad 1990) and others have been sequenced. Organization of the operon and predicted amino acid sequences of these four polypeptides are highly conserved in these organisms except in *Euglena* (Cushman *et al.* 1988a, b) and *Chlamydomonas* (Mor *et al.* 1995). In *Euglena*, the four genes are cotranscribed with *psaA* and *psaB*, which encode the reaction-centre polypeptides of photosystem I, as *psaA-psaB-psbE-psbF-psbL-psbJ* transcript (Cushman *et al.* 1988a, b). Recently, it was reported that the *psbE* and *psbF* genes of *Chlamydomonas reinhardtii* are separated by 8 kbp and are encoded on opposite DNA strands yielding separate transcripts for these two genes (Mor *et al.* 1995).

In this report we present the nucleotide sequence and analysis of *psbE-F-L-J* operon from the chloroplast genome of a tree species, *Populus deltoides*, commonly known as poplar. Among trees, only the chloroplast genome of blackpine has been sequenced fully (Wakasugi *et al.* 1994).

2. Materials and methods

2.1 *Cloning of psbE-F-L-J operon*

Chloroplast DNA (cpDNA) was isolated and purified from green leaves of *Populus deltoides* L. clone D121 as described by Nath *et al.* (1993). A library of *Hin*dIII-digested cpDNA was constructed in pBluescriptIIKS(+) phagemid and transformed in *E. coli XL1 Blue* (Trivedi *et al.* 1994). Heterologous spinach probe carrying *psbE* gene taken out as a 194-bp *Eco*RI–*Hin*dIII fragment from clone $pCYb_{559/1}$ (a gift from Prof R.G. Herrmann, Botanisches Institute der Universität, Münich, Germany) was radiolabelled by random primers (Sambrook *et al.* 1989) and used as a probe. Recombinant clones carrying *psbE* region were identified by colony hybridization (Sambrook *et al.* 1989).

2.2 *Subcloning and sequence analysis*

General molecular-genetic methods were carried out as described by Sambrook *et al.* (1989). The insert in recombinant clone pPCYT was subcloned in pBluescriptIIKS(+) using *Xba*I, *Eco*RI, *Sac*I, *Apa*I and *Dra*II restriction enzymes. All the subclones were sequenced from both the ends. A 3·5-kbp *Hin*dIII–*Xba*I subfragment (in pPXCYT) comprising complete *psbE-F-L-J* operon was selected for further studies. Unidirectional nested deletions of pPXCYT were prepared using exonuclease III/mungbean system (Stratagene) according to manufacturer's instructions. Sequencing reactions of double-stranded plasmids were carried out according to manufacturer's instructions using ^{35}S-αdATP (Amersham) and T7 sequencing kit (Pharmacia). The reaction products were resolved on urea–acrylamide gel and autoradiographed for determining the sequence. Alternatively the automated DNA sequencing system (373, ABI) was used for obtaining the sequence using dye terminator cycle sequencing kit (Perkin Elmer).

2.3 *Nucleotide substitution analysis*

Pairwise multiple alignments of nucleotide sequences in coding regions were done using Clustal software in PC/GENE release 14.0 (Intelligenetics Inc., Mountain View, USA) and sites of nucleotide substitutions were analysed. Nucleotide substitutions were divided into two categories: (i) synonymous substitutions (SS), i.e. nucleotide sequence changes that result in synonymous change at codon level without changing the amino acid, and (ii) nonsynonymous substitutions (NS), i.e. changes in nucleotide sequence that result in change at the amino acid level.

2.4 *RNA isolation and Northern hybridizations*

Total leaf RNA was prepared from leaves of *Populus deltoides*. Leaves were harvested and frozen in liquid nitrogen and RNA was extracted by guanidine isothiocyanate method (Bate *et al.* 1991). RNA was separated on a denaturating 1·3% agarose MOPS–formaldehyde gel and transferred to Zeta-probe membrane (Biorad). The RNA gel blots were hybridized with DNA probes labelled by random priming (Sambrook *et al.* 1989) in 250 mM sodium phosphate (pH 7·2), 7% SDS, 1 mM EDTA and 50% formamide at 42°C for 16 h. All probes used for Northern hybridizations were homologous probes from poplar, except $pCYb_{559/1}$ from spinach.

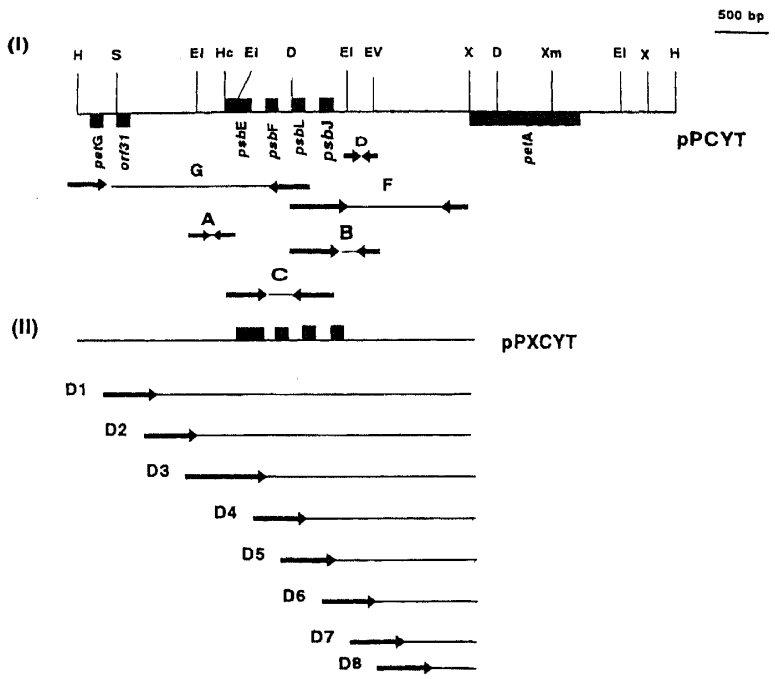

Figure 1. (I) Restriction endonuclease and genetic map of the *Hin*dIII insert in clone pPCYT along with the subclones (A–G). (II) Genetic map of subclone pPXCYT along with the deletion clones (D1–D8). The direction and the length of arrows in the subclones and deletions represent the direction of sequencing reaction carried out and the number of nucleotides read. D, *Dra*II; EI, *Eco*RI; EV, *Eco*RV; Hc, *Hin*cII; H, *Hin*dIII; S, *Spe*I; X, *Xba*I; Xm, *Xmn*I.

3. Results and discussion

3.1 *Cloning and organization of the psbE-F-L-J operon*

The heterologous *psbE* probe from spinach hybridized to a clone containing 5·5-kbp insert in cpDNA library of *Populus deltoides*. It was designated as pPCYT. Both the ends of this clone were sequenced and compared with EMBL nucleotide sequence database release 14.0 from Intelligenetics Inc., USA. At one end, it showed homology to the *petG* coding region while the other end resembled upstream region of *petA*. Since in most of the organisms *petA* and ORF31-*petG*-ORF42 operons have been reported to flank the genes for cyt b_{559}, the insert in pPCYT was examined in further detail.

Restriction endonuclease map was established for the 5·5-kbp insert in pPCYT [(figure 1(I)]. It was subcloned using *Xba*I, *Eco*RI, *Sac*I, *Apa*I and *Dra*II restriction enzymes. Each subclone was sequenced from both the ends. The sequences obtained from the subclones and the deletion clones D3 to D7 [figure 1(I and II)] led to identification of the *psbE-F-L-J* operon. The nucleotide sequence (figure 2) was submitted to EMBL database under Accession No. X89651.

Analysis of the reading frames suggested that the *psbE-F-L-J* operon was located in between but transcribed divergently with respect to ORF31-*petG* and *petA* operons [figure 1(I)], as reported for other higher plants and cyanobacteria. *Euglena gracilis* is the only known exception in which *psbE-F-L-J* locus is unique in two respects. First, the four genes are cotranscribed with the *psaA* and *psaB* genes. Secondly, the hexacistronic operon *psaA-psaB-psbE-psbF-psbL-psbJ* contains at least 12 introns, three in *psaA*, six in *psaB*, two in *psbE* and one or more in *psbF* (Cushman *et al.* 1988a, b). In *Chlamydomonas reinhardtii, psbE* and *psbF* genes are separated by 8-kbp and are encoded on opposite strands and form separate transcription units (Mor *et al.* 1995).

3.2 *Analysis of the nucleotide substitutions in the coding regions*

An analysis of the SS and NS in *psbE, psbF, psbL* and *psbJ* coding regions among the various reported sequences is shown in figure 3. In all cases, SS are distributed throughout the entire length of the genes. The rate of SS is reported to be related to codon usage (Sharp and Li 1987; Shields *et al.* 1988; Sharp 1991) and sequence composition (Moriyama and Gojobori 1992). Codon usage in *E. coli* as well as in *Saccharomyces cerevisiae* is correlated with tRNA abundance (Ikemura 1985). The degree to which codon usage is biased in a gene has been suggested to correspond to the level of expression of that gene. Selection at codon level is suggested to influence translational efficiency (Dix and Thompson 1989). The variation in the rate of SS is suggested to result from the level of gene expression (Sharp and Li 1987; Sharp 1991). Highly expressed genes of the *Marchantia polymorpha* chloroplast genome have been reported to show bias for certain synonymous codons (Morton 1994).

The frequency of NS varies considerably across the genes and occurs at lower rate compared to SS within a given genome (Li *et al.* 1985). Studies on mammalian genes have shown a great deal of variation in the rate of NS (Li *et al.* 1985) resulting from differences in selective constraints on amino acid replacement across the genes. Our analysis of the reading frames in *psbE-F-L-J* operon shows that NS occur at a lower frequency than SS in all four genes. Similar results were obtained (data not given) when reading frames of longer genes (like *psbA, psbD* and *rbcL*) were compared. The rate of

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AGAAATCAAGGAAACACCTTTTGCTTCGAATACAAGAACAAGAATACACACATTCAGAATATTGATTCTTACAAT
TATTTTTTCGCTGTTCTCTCGAATATTCTCTACTGCCAGTACTTGTTTCTGGACAACTAAGCAATTCCTTAAAAT
GAAAAAAGGATTTCAACAAAAAACTCTTATTCTACAAGTACGAAGGGGAGGGGGTTAGATTTCGCATCTAATTGA
ATTGTGAATTCGGGGAATAGGCTAATCTCTTTTATGAATTTTTATTATTAATTATTAGAAAACAACCCGTCAAAT
TCACATTTTTCTCTAATCTATGGTACACGGTTCTACAGTGACAAGAAAAAGAAAAGACTAAAGAAATTATATAGG
ACTTCGTTTCGAGACTGATTGGAGTTGCGTTGCTGCGTCAGAAGAAGGATAGCTATACTGATTTGGTATACTTTA
AAGAAACCCTCGGTACGATATTGACGATCTCACAAAGATTGAATTTCAGTGAATTTTCATTTACTGATCTCGTCT
                                                    psbE
                                                      M   S
TTTACGGAATCGATCCCCTTTTGACTGTACAAGAATATTGGAGCTCGC    ATG TCT
 G    S    T    G    E    R    S    F    A    D    I    I
GGA  AGC  ACA  GGA  GAA  CGT  TCT  TTT  GCT  GAT  ATT  ATT
 T    S    I    R    Y    W    V    I    H    S    I    T
ACC  AGT  ATT  CGA  TAT  TGG  GTC  ATT  CAT  AGC  ATT  ACT
 I    P    S    L    F    I    A    G    W    L    F    V
ATA  CCT  TCC  CTA  TTC  ATT  GCA  GGT  TGG  TTA  TTC  GTC
 S    T    G    L    A    Y    D    V    F    G    S    P
AGC  ACA  GGT  TTA  GCT  TAT  GAT  GTA  TTT  GGA  AGC  CCT
 R    P    N    E    Y    F    T    E    S    R    Q    G
CGT  CCA  AAT  GAA  TAT  TTT  ACA  GAG  AGC  CGA  CAA  GGA
 I    P    L    I    T    G    R    F    D    P    L    E
ATT  CCC  TTA  ATA  ACT  GGC  CGT  TTT  GAT  CCT  TTG  GAA
 Q    L    D    E    F    S    R    S    F
CAA  CTT  GAT  GAA  TTT  AGT  AGA  TCT  TTT  TAG GTTAGGAGG
     psbF
       M    T    I    D    R    T    Y    P    I    F    T
GCCCA ATG  ACT  ATA  GAT  CGA  ACC  TAT  CCA  ATT  TTT  ACA
  V    R    W    L    A    V    H    G    L    A    V    P
 GTG  CGA  TGG  CTT  GCT  GTT  CAC  GGA  CTA  GCT  GTA  CCT
  T    V    S    F    L    G    S    I    S    A    M    Q
 ACC  GTT  TCT  TTT  TTG  GGG  TCA  ATA  TCA  GCA  ATG  CAG
  F    I    Q    R
 TTC  ATC  CAA  CGA  TAA   ACCTAATCTGAATTAATATTAATTATAAAGCT
psbL
 M    T    Q    S    N    P    N    E    Q    N    V    E
ATG  ACA  CAA  TCA  AAC  CCG  AAC  GAA  CAA  AAC  GTT  GAA
 L    N    R    T    S    L    Y    W    G    L    L    L
TTG  AAT  CGT  ACC  AGT  CTT  TAC  TGG  GGG  TTA  TTA  CTC
 I    F    V    L    A    V    L    F    S    N    Y    F
ATT  TTT  GTA  CTT  GCT  GTT  TTA  TTT  TCT  AAT  TAT  TTC
 F    N
TTT  AAT  TAA   TATTAAAATTAAATTTATTTCTTTAAATTAATATTTAATTAATAATTAAATAT
TAATTAAAAAAGGAAAAGAGAGTATTAGAATAATAAATAAGAGCAGAAATTCTCTTATCTCGTCGGAAGGATA
TCATCACATAATTATCTATGACTGTTCATGTCTCTAGCAGGACCACTTGATTAAATGTGGAGGAATGGGATAA
psbJ
 M    A    D    T    T    G    R    I    P    L    W    I
ATG  GCC  GAT  ACT  ACT  GGA  AGA  ATT  CCT  CTT  TGG  ATA
 I    S    T    V    T    G    I    P    V    I    G    L
ATA  AGT  ACT  GTA  ACT  GGT  ATT  CCT  GTG  ATC  GGT  TTA
 I    S    I    F    F    Y    G    S    Y    S    G    L
ATA  AGT  ATT  TTC  TTT  TAT  GGC  TCC  TAT  TCC  GGC  TTG
 G    S    S    L
GGT  TCA  TCC  CTG  TAG  TAATAGGATGAACTGAGTTTGTAGACATGAAAGCGTAAGAACTCA
ACGGCATCCCCGTCGAATCAGACGGAAAAAAGGGGGCGCTGCTGAGTTCTTAATAATCAGTAATACTTTATTCGT
ATAAATGACAAAATGAATTCCTTCGTCTGTATTTCTATCTTTGGTGGTCGGAATTCATGGGGGCCCAGCGTGACT
GGCAAAGCAATACAAAGTACGGAAAGCCAGTCATGATTAAAGGTGGAATTC
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Figure 2. Nucleotide sequence of the 1672-bp portion of pPCYT containing *psbE-psbF-psbL-psbJ* operon. The deduced amino acid sequence is shown above the nucleotide sequence. Underlined portions of the sequence are the putative ribosome binding sites (RBS). A sequence motif resembling putative RBS for the *psbL* gene was not found in the nucleotide sequence. The bold letters represent putative promoter sequence (− 10 and − 35) motifs.

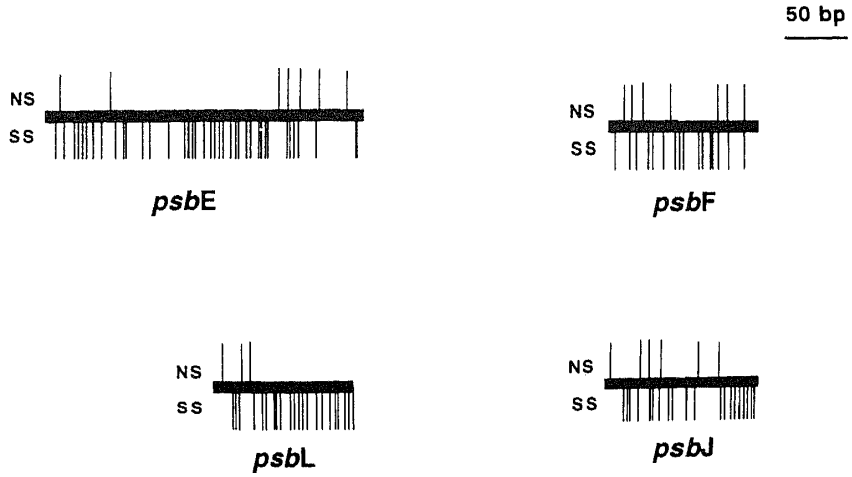

Figure 3. Distribution of synonymous (SS) and nonsynonymous (NS) nucleotide substitutions in the *psbE*, *psbF*, *psbL* and *psbJ* genes based on pairwise alignments of nucleotide and amino acid sequences.

NS is highly variable across the genes. In *psbE* and *psbL*, NS are located at C-terminal and/or N-terminal regions of the polypeptides. In *psbJ*, NS are present along entire length of the gene. In *psbF*, except two (position 18 and 23) all other NS are located on either N-terminal or C-terminal region. None of the NS are present within the haem-binding site and transmembrane α-helical region of the *psbE* and *psbF* genes (figure 3). Further, the codon 26 (UCU in the present case) of *psbF* gene, which encodes serine, has been shown to be edited to UUU giving phenylalanine in several other species (Bock *et al.* 1993). We presume that in poplar also this codon (UCU) will be edited to give Phe (UUU). The Phe at this position of the β-subunit has been demonstrated to be indispensable from the functional point of view (Bock *et al.* 1994).

NS were further analysed for replacement with similar residues, viz. those that can interchange with each other without modifying the biological activity of a protein (Myers and Miller 1988). The following groups have been considered as similar residues (S); (i) A, S, T, G and P, (ii) D and E, (iii) N and Q, (iv) R and K, (v) I, L, M and V, and (vi) F, Y, and W. Residues which belong to different groups but still do not differ in charge were considered semiconserved (Sc). Those differing in group as well as charge were considered as non-conserved (N). In *psbE* gene, out of the seven NS, only two sites (29 and 61) represent non-conserved sites and may be indicative of the positions that can tolerate changes without affecting functional integrity of the polypeptide. Other sites are either Sc or S and therefore may not cause a drastic perturbation (table 1).

In *psbF*, out of the 7 NS sites, four are S, one Sc and two are N. N sites are present on the N-terminal region of the polypeptide. In *psbL* two changes are Sc and one is N. Similarly in *psbJ*, two sites are S, three are Sc and one is N. N site lies on the N-terminal (table 1) in both the cases. The location of NS sites may be indicative of the regions in the polypeptides that cause very little structural and functional perturbations.

3.3 *Analysis of noncoding regions*

In contrast to the coding regions, the noncoding upstream, downstream and spacer regions of *psbE-F-L-J* operon are highly variable and show low level of homology

Table 1. List of nonsynonymous substitutions in *psbE, psbF, psbL*and *psbJ* genes.

Gene	Amino acid position in putative polypeptide	Amino acid substitutions	Nature of substitutions
psbE	3	G, S, T, N	Sc
	29	F, M, S	N
	45	V, I, A	Sc
	61	E, Q, G	N
	62	V, L, I	S
	66	N, Q, S, T	Sc
	67	Q, E, G, D	N
psbF	3	I, T, N, Q	Sc
	5	R, K, D	N
	6	N, D, T	N
	18	G, A, T	S
	23	TSA	S
	28	I, L, V	S
	32	S, A, T	S
psbL	4	P, S,T	Sc
	8	K, D, E	N
	10	S, T, V, N	Sc
psbJ	3	N, D	N
	14	L, A, G, S	Sc
	17	I, A, T	Sc
	20	A, L, V, P	Sc
	22	L, I, V	S
	24	L, I, V	S

Translational frames of *psbE, psbF, psbL*and *psbJ* genes from *P. deltoides, N. tabacum, S. oleracea, M. crystallinum, P. sativum, O. sativa, H. vulgare, Z. mays, P. thunbergii* and *M. polymorpha* were compared to score the amino acid positions with different substitutions. For S, Sc and N refer text.

(50–70%) among different photosynthetic organisms. The upstream region has high AT content and possesses putative −10 and −35 sequence motifs, which may be promoter elements (figure 2). Downstream of the stop codon of *psbJ* gene is an AT-rich region capable of forming a stem–loop structure. Stem–loop structures at 3' end of chloroplastic transcripts have been suggested to confer stability and/or help in processing of chloroplastic mRNA (Stern *et al.* 1989; Schuster and Gruissem 1991).

Spacer regions between adjacent genes of the *psbE-F-L-J* operon differ in length when compared among different plant species. For example, the spacer region between the stop codon of *psbE* and initiation codon of *psbF* gene in poplar chloroplast genome has a repeat of the (TTAGG) pentanucleotide as compared to tobacco (figure 2). This insertion could arise as a result of slipped strand mispairing during replication, as has been reported for *rpoC2* gene of rice (Cummings *et al.* 1994). The spacer region between stop codon of *psbF* and the initiation codon of *psbL* has an insertion of 10 nucleotides in poplar as compared to tobacco (figure 2). This insertion in poplar is not a duplication of neighbouring sequence. Spacer regions in liverwort are shorter than those in rice and tobacco. However, tobacco and poplar have longer spacer regions than rice. Such differences in spacer and intergenic regions may account partly for the differences in

Table 2. RNA editing within *psbF* and *psbL* genes of different dicotyledonous plant species.

Species	*psbF* UCU to UUU (Ser to Phe)	*psbL* ACG to AUG (Ser to Met)
Populus deltoides	+ (likely)	−
Spinacia oleracea	+	+
Pisum sativum	+	−
Oenothera hookeri	+	n.d.
Nicotiana tabacum	−	+

n.d., Not detected.

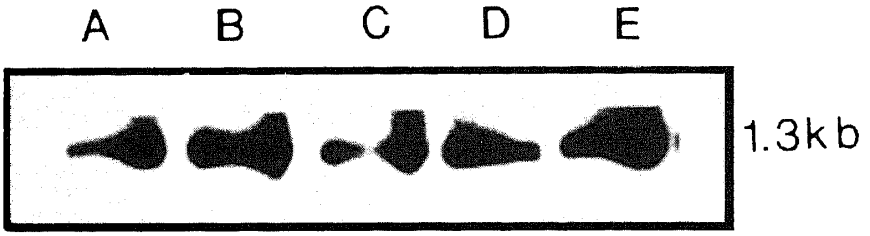

Figure 4. Northern analysis of *psbE-F-L-J* operon using subclones from different regions (A–D) of the operon (for details refer figure 1(I)) and heterologous probe from spinach (E).

genome size among different chloroplasts (Shimada and Sugiura 1991). It remains to be seen whether these insertions have any functional role.

3.4 *Editing pattern*

In *psbE-F-L-J* operon two editing sites (C to U) have been demonstrated in different plant species. Of these, one editing site resides in *psbF* gene restoring the conserved phenylalanine (UUU) from serine (UCU) at position 26 of β-subunit of cyt b_{559} (Bock *et al.* 1993). It has been shown that lack of editing from UCU to UUU in transgenic tobacco results in slower growth, lower chlorophyll content and high chlorophyll fluorescence, a phenotype characteristic of a photosynthetic mutant (Bock *et al.* 1994).

The second editing site present in *psbL* gene in many plants relates to restoration of AUG, the initiation codon, from ACG. In poplar, however, editing of this site is not needed as ATG itself is present in the nucleotide sequence (figure 2). A comparison of poplar with other plant sequences where the above mentioned potential editing sites exist show that poplar is identical to pea in this respect (table 2).

3.5 *The psbE-F-L-J gene cluster gives rise to a single tetracistronic transcript*

A 1·3-kb transcript corresponding to *psbE-F-L-J* operon was detected in *Populus deltoides*. There was no indication of smaller transcripts that should be present if there was a post-transcriptional processing (figure 4). It agrees well with the reported existence of a tetracistronic message of 1·1 kb in spinach (Westhoff *et al.* 1985), *Oenothera* (Carillo *et al.* 1986) and wheat (Webber *et al.* 1989) and of 1·3 kb in pea

(Woodbury *et al.* 1988). Such differences in transcript size among different plants can be accounted for by variable length of spacer regions and the differences in upstream and downstream regions. Use of the subfragments (A–D, figure 4) of the clone pPXCYT from different regions of the operon and a heterologous DNA probe from spinach (E, figure 4) provided no evidence indicative of internal transcription initiation sites and processing of the transcript. The products of all the genes of *psbE-F-L-J* operon have been shown to be associated with the PSII complex. It is therefore expected that the four polypeptides are required in stoichiometric amounts and the translational products of this single transcript can meet this requirement.

3.6 *Differential accumulation of psbE-F-L-J transcript during leaf development*

Leaves from different nodal positions were taken as representatives of different developmental stages and Northern blots were prepared with total RNA prepared in two different seasons, summer and winter. Northern hybridization data revealed quantitative increase in *psbE-F-L-J* transcript level in mature leaves as compared to young leaves (figure 5) in both seasons. Steady-state mRNA levels of nuclear (*rbcS*, *cabS*) and plastid (*rbcL*, *psbA*)-encoded genes have earlier been reported for tomato leaves at different developmental stages. The *rbcS* and *cab* transcript levels were high in 4-cm (young) leaves, while transcripts of *rbcL* and *psbA* accumulated to high levels in 6-cm (mature) tomato leaves (Piechulla 1988). In *Phaseolus vulgaris* also dot-blot and Northern hybridization studies indicated that the transcript levels for *cab* and *rbcS* decreased with maturation of leaves while the *psbA* transcript was present in higher proportion of total RNA in 14–21- and 28-day-old tissue than in 7-day-old tissue (Bate *et al.* 1991). It is notable that the transcript levels for the chloroplast genes *rrn* and *rbcL* remained in constant proportion of total RNA throughout senescence (Bate *et al.* 1991). In *Populus deltoides* there was no significant change in transcript level of 23S rRNA in mature leaves as compared to young leaves (figure 5).

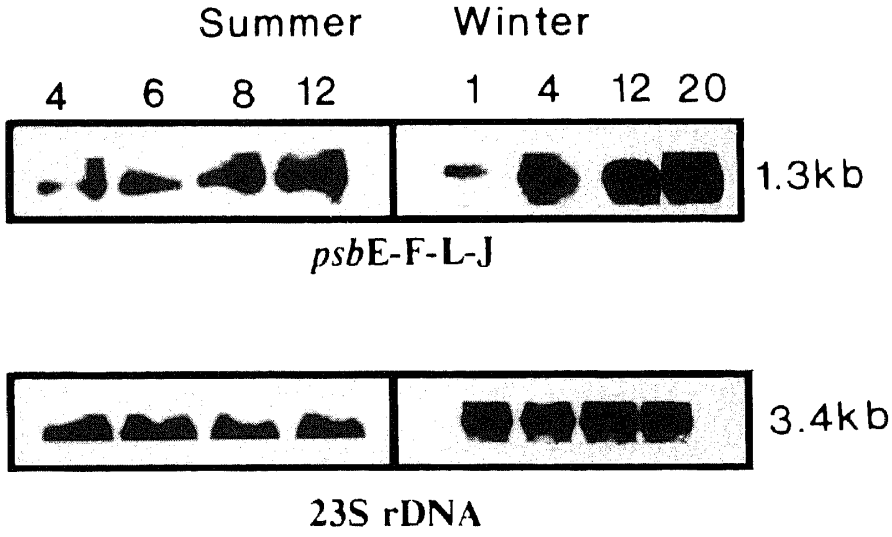

Figure 5. Northern analysis using RNA isolated from different developmental stages of leaves (from nodal position 1 to 20) in two different seasons, namely summer and winter. Homologous 23S rDNA and *psbE-F-L-J* (subclone C in figure 1) were used as probes. Sizes of the transcripts are shown in kb.

During maturation of chloroplasts, a decrease in overall transcriptional activity was detected in barley (Krupinska 1992), spinach (Deng and Gruissem 1987), tobacco (Wollgiehn *et al.* 1976) and wheat (Patterson and Smillie 1971). The decrease in overall transcriptional activity is mainly due to differential decrease in *rrn* transcription (Krupinska 1992). An opposite course of transcriptional activity during chloroplast maturation was reported for *psbD* and *psbA* genes (Krupinska 1992). Differential accumulation of the transcript in poplar and in other plants might be due to differential levels of synthesis and stability of the transcripts (Kim *et al.* 1993). Development-dependent change in RNA polymerase activity can lead to initiation of transcription from new loci with the help of some developmentally induced transcription factor (Haley and Bogorad 1990) and may also be responsible for differential accumulation.

Our studies on *psbE-F-L-J* operon suggest that the organization and nucleotide and deduced amino acid sequence of *Populus deltoides* are highly homologous to that in the other land plants studied. However, noncoding and spacer regions are variable with respect to other plants. This operon gives rise to a single tetracistronic transcript, which shows quantitative increase in mature leaves.

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