Structure and expression of a nuclear gene for the PSI-D subunit of photosystem I in *Nicotiana sylvestris*

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**Abstract**

The PSI-D subunit is the ferredoxin-binding site of photosystem I, and is encoded by the nuclear gene *psaD*. We isolated a *psaD* genomic clone from *Nicotiana sylvestris*, by screening a genomic library with a *psaD* cDNA which we previously cloned from *N. sylvestris* (Yamamoto et al., Plant Mol Biol 17:1251, 1991). Nucleotide sequence analysis revealed that this genomic clone contains a *psaD* gene, which does not correspond to the *psaD* cDNA, so we designated these genes *psaDb* and *psaDa*, respectively. The *psaDb* clone encodes a protein of 214 amino acids uninterrupted by introns. The N-terminal sequence determined for the *N. sylvestris* PSI-D protein encoded by *psaDb* begins at the 49th residue. The products of *psaDa* and *psaDb* share 82.7% and 79.5% identity at the amino acid and nucleotide levels, respectively. Genomic Southern analysis showed that two copies of *psaD* are present in the *N. sylvestris* genome. Ribonuclease protection assays and immunoblot analysis in *N. sylvestris* indicate that both genes are expressed in leaves, stems and flower buds, but neither is expressed in roots. During leaf development, the ratio of *psaDb* to *psaDa* mRNA increases from 0.12 in leaf buds to 0.36 in mature leaves. The relative abundance of the corresponding proteins decreased over the same developmental period. These results indicate that differential regulation mechanisms control *psaDa* and *psaDb* expression at both the mRNA and protein levels during leaf development.

**Introduction**

Photosystem I (PSI) mediates photosynthetic electron transfer from plastocyanin to ferredoxin. PSI is a multiprotein complex in the thylakoid membranes of chloroplasts, composed of at least 12 subunits, designated PSI-A through PSI-L. The genes encoding these proteins are designated *psaA* through *psaL*, respectively [26, 31]. PSI-A, PSI-B, PSI-C, PSI-I and PSI-J are encoded in the chloroplast genome, while the remaining seven subunits are nuclear-encoded [11, 26, 31].

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number D13718.
PSI-D is located on the stromal surface of PSI [18, 25], and is the binding site of ferredoxin [39, 40]. The primary structure of the PSI-D subunit has been determined in several plant species by cDNA analysis [12, 13, 17, 22, 38]. *Nicotiana* species have two PSI-D isomers per genome, which differ from each other in N-terminal (partial) amino acid sequence as well as in apparent molecular mass, according to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis [23]. *Nicotiana sylvestris*, a diploid species, has two PSI-D isoforms with apparent molecular masses of 19 kDa and 17.5 kDa. We have designated these PSI-D1 and PSI-D2, respectively [23]. The relative abundance of PSI-D1 to PSI-D2 is very low, hence most PSI complexes in the plant are thought to contain PSI-D2 instead of PSI-D1. We previously isolated *psaD* cDNA clones which encode PSI-D2 of *N. sylvestris*, and designated the corresponding gene *psaDa* [38].

It is well known that the small subunit of ribulose 1,5-bisphosphate carboxylase (rbcS) and the chlorophyll a/b-binding protein (Cab) are encoded by multi-gene families in the nuclear genome [4, 5, 21, 29]. The expression of each gene in a given family is differentially regulated with respect to the others in response to plant development or environmental stimuli [34]. Ferredoxin and the 33 kDa protein of the oxygen-evolving complex are also encoded by multi-gene families in the nuclear genome [10, 37]. However, little is known about the organization of the photosystem gene family within the nuclear genome. Clarification of the structure and organization of the nuclear genes encoding the PSI subunits is a prerequisite for analysis of the molecular mechanisms which control PSI biogenesis.

Many studies of thylakoid protein structure and photosynthetic gene expression have been carried out in *Nicotiana tabacum*. However, *N. tabacum* is an allotetraploid, with a very large genome and multiple copies of most genes, which complicates molecular analysis and studies of gene structure. *N. sylvestris* is an autogamous diploid species, which is the maternal ancestor of *N. tabacum* [24] and is readily transformable. Therefore, we have concentrated our studies in *N. sylvestris*. We have isolated a genomic clone containing the second *psaD* gene from *N. sylvestris*, and characterized this gene in comparison with the *psaDa* gene, mentioned above.

**Materials and methods**

**Plant material**

*Nicotiana sylvestris* was grown at 25 °C in a greenhouse. Root tissues were harvested from hydroponically grown plants.

**DNA and RNA preparation**

Nuclear DNA was isolated and purified by CsCl-EtBr ultracentrifugation according to Jofuku and Goldberg [14].

Total RNA was prepared according to the method of Piechulla et al. [28], from leaf buds of less than 3 cm in longitudinal axis, developing leaves of 5–10 cm, mature leaves of 20–30 cm, stems, flower buds and roots. These organs were harvested in the afternoon on fine days.

**Genomic cloning and sequence analysis**

An *N. sylvestris* lambda dash genomic library [20] was screened with the insert of a *psaDa* cDNA clone, yaDC17 [38], which was labeled with [α-32P]dCTP by the random hexanucleotide-priming method [30]. A 5.7 kb Xba I fragment of the positive clone was subcloned into pBluescript SKII +, and subjected to DNA sequence analysis.

**Mapping of the transcription start site**

Primer extension mapping was carried out essentially according to Sambrook *et al.* [30]. An end-labelled primer was hybridized with 20 μg of total RNA from *N. sylvestris*, and elongated with