**Plant Gene Register**

**Nucleotide Sequence of an Arabidopsis cDNA for Geranylgeranyl Pyrophosphate Synthase**

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GGPS is an enzyme involved in the early steps of isoprenoid synthesis in plants. The bell pepper (Capsicum annuum) GGPS catalyzes the stepwise addition of isopentenyl PPi, first to dimethylallyl PPi, second to geranyl PPi, and finally to farnesyl PPi to form geranylgeranyl PPi (Kuntz et al., 1992). Geranylgeranyl PPi is utilized by plants for many compounds such as carotenoids, Chls, and phylloquinones and as a precursor for GA3. A plant GGPS cDNA from Capsicum ripening fruit has been cloned and sequenced, but only the deduced amino acid sequence has been published (Kuntz et al., 1992).

To obtain a GGPS cDNA, we have cloned, by amplification using the PCR, a gene fragment for GGPS from Arabidopsis using degenerate oligonucleotides based on conservation of amino acids among several GGPSs (Carattoli et al., 1991; Michalowski et al., 1991; Kuntz et al., 1992; Math et al., 1992). An upstream oligonucleotide, 5'-GCIGCITG(T/C)GCIGTIGA(A/G)ATG-3' (I = inosine), corresponding to the peptide sequence DDILDVT, were used to amplify Arabidopsis genomic DNA. Upon electrophoresis of the resulting PCR products, a single band of about 450 bp was observed; it was purified and subcloned into SmaI-cut pBluescript using a procedure developed in our laboratory. This fragment was used to screen a cDNA library constructed from norflurazon-treated seedlings (Table I). One plaque hybridizing to the probe was purified and the cDNA insert sequenced. Analysis of the sequence revealed an open reading frame encoding a protein of 371 amino acids. Alignment of this protein to GGPSs from Erwinia uredovora and Rhodobacter capsulatus suggests an N-terminal transit peptide of 76 amino acids. Comparison of this protein sequence to the protein sequence of GGPS from pepper showed 84.5% similarity and 73.5% identity between the putative mature peptides and, unsurprisingly, very little similarity or identity between the putative transit peptides. Sequence differences between the cDNA and the genomic fragment indicate the existence of more than one GGPS gene in Arabidopsis. We propose naming this gene GGPS1. The genomic fragment has been mapped to the lower arm of chromosome 2, whereas the cDNA has shown no polymorphisms with identical genomic blots (Reiter et al., 1992).

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**Table I. Characteristics of an A. thaliana GGPS cDNA**

| Organism: Arabidopsis thaliana (L.) Heynh., WS ecotype, Brassicaceae. |
| Enzyme, Function: GGPS, isoprenoid biosynthesis. |
| Source: Custom cDNA library in XZAPII constructed by Clontech from norflurazon-treated seedlings and screened by DNA hybridization. |
| Cloning Strategy: PCR of genomic DNA using degenerate oligonucleotides based on conserved amino acid sequences of GGPS and subsequent hybridization to the cDNA library. |
| Techniques: Genomic DNA was amplified in a Perkin-Elmer 9600 thermocycler for 35 cycles using components of a PCR kit (Perkin-Elmer Cetus, Norwalk, CT) and the following program: denaturation, 92°C, 0.5 min, ramp time 0.5 min; annealing, 50°C, 0.5 min, ramp time 2 min; extension, 72°C, 3 min, ramp time 1 min; final extension, 72°C, 8 min. The resulting PCR product was treated with the large fragment of T4 polymerase and with polynucleotide kinase, gel purified using Spin-X (Costar, Cambridge, MA), and ligated into SmaI-cut pBluescript KS+. The sequence of the subcloned insert and cDNA was determined by deoxy sequencing of double-stranded DNA using a Sequenase kit (United States Biochemical). |
| Characteristics of cDNA: cDNA of 1242 nucleotides containing a 5' untranslated region of 29 bp and a 3' untranslated region of 100 bp. No poly(A) tail observed. |

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LITERATURE CITED


