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Plant Gene Register

Nucleotide Sequence of a cDNA Clone Encoding Caffeoyl-Coenzyme A 3-*O*-Methyltransferase of *Stellaria longipes* (Caryophyllaceae)[1]

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Phenylpropanoid metabolism consists of a range of pathways leading to various defense-related products. These products are fundamental to plant growth and to differentiation under normal environments, as well as during stress (Bowles, 1990). The involvement of phenylpropanoid metabolism in adaptive responses of plants to various environmental stimuli such as wounding, irradiation, and infection has been extensively studied (Bowles, 1990; Dixon and Lamb, 1990). Among many enzymes involved in the phenylpropanoid pathways, CCoAMT (EC 2.1.1.104) is an enzyme specific for the substrate *trans*-caffeoyl-CoA (Kneusel et al., 1989; Pakusch et al., 1991) and catalyzes the synthesis of *trans*-feruloyl-CoA. The latter product is a necessary intermediate in the biosynthesis of coniferyl alcohol—one of three precursors (along with 4-coumaryl and sinapyl alcohols) for lignin formation within the plant cell wall. Among other functions, lignin is believed to contribute to plant defense.

CCoAMT activity or mRNA transcription has been detected in cell-suspension cultures of parsley (*Petroselinum crispum*), Bishop's weed (*Ammi majus*), carnation (*Dianthus caryophyllus*), safflower (*Carthamus tinctorius*) (Pakusch et al., 1991; Schmitt et al., 1991), and carrot (*Daucus carota*) (Kuhnl et al., 1989). The CCoAMT cDNA sequence has been reported only from parsley (Schmitt et al., 1991). The native CCoAMT enzyme in parsley is a homodimer, existing at a moderately high level in tissues undergoing normal growth. Another *O*-methyltransferase of lignin biosynthesis, *S*-adenosyl-L-Met:caffeic acid 3-*O*-methyltransferase (EC 2.1.1.6), has been extensively investigated in many plants (Gowri et al., 1991; Collazo et al., 1992). However, it is distinctly different from CCoAMT and does not depend on a CoA-ester substrate (Pakusch et al., 1991). There is no published information about the CCoAMT enzyme or the gene that encodes it, except for the study of parsley (Schmitt et al., 1991). Here we report the cDNA clone encoding CCoAMT of *Stellaria longipes*.

S. longipes Goldie (Caryophyllaceae) is a dicotyledonous herbaceous perennial that exhibits marvelous phenotypic plasticity in response to different environmental conditions (Chinnappa and Morton, 1984; Zhang and Chinnappa, 1994). As part of an investigation of the molecular basis of population differentiation and adaptation, a cDNA clone

Table I. *Characterization of a cDNA encoding CCoAMT of S. longipes*

Organism:
Stellaria longipes Goldie (Caryophyllaceae; common name long-stalked chick weed; genotype 5D).

Source:
cDNA library in λZAP II vector (Stratagene) constructed from leaf poly(A)$^+$ RNA.

Techniques:
Sequencing of double-stranded plasmid clone c9 (pBluescript in *Escherichia coli* strain XL-1 Blue); synthetic oligonucleotides as primers and dideoxy sequencing of both strands.

Method of Identification:
Sequence comparison shows high similarity to CCoAMT (EC 2.1.1.104) of parsley (Schmitt et al., 1991) at both nucleotide and deduced amino acid levels.

Features of the cDNA Clone:
The c9 sequence contains 17 nucleotides of 5′ noncoding region, 726 nucleotides of coding region, and 270 nucleotides of 3′ noncoding region. A putative polyadenylation signal sequence, AATAAA, appears 107 bp downstream of the termination codon TGA.

Features of the Deduced Protein:
The open reading frame predicts a polypeptide of 241 amino acids with a calculated molecular mass of 26.7 kD and estimated pI of 5.2. Analysis of the deduced amino acid sequences show that both the *S. longipes* c9 protein and the parsley CCoAMT share very similar structural features throughout the sequence, such as hydrophilicity, surface probability, backbone chain flexibility, amphiphilicity, antigenicity, and secondary structure. Two conserved Cys's are located in residues 209 and 237, respectively, which are suggested to be involved in bridging the native dimer and/or catalytic activity (Schmitt et al., 1991).

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Abbreviation: CCoAMT, *S*-adenosyl-L-methionine:*trans*-caffeoyl-CoA 3-*O*-methyltransferase.

encoding CCoAMT-like protein was isolated from a cDNA library of *S. longipes* and analyzed (Table I). Northern blot hybridization showed that the CCoAMT mRNA level from stems was higher than from leaves and much higher than from roots, indicative of a certain degree of tissue-specific gene expression of CCoAMT occurring in *S. longipes*. PCR analysis demonstrated that the CCoAMT cDNA corresponding gene did not contain an intron in its coding region. The CCoAMT gene family consists of two members in the *S. longipes* genome.

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