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Plant Gene Register

A Full-Length cDNA Encoding 1-Aminocyclopropane-1-Carboxylate Synthase from Apple[1]

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Ethylene plays an important role in many plant processes including fruit ripening. The two key enzymes in the ethylene biosynthetic pathway are ACC synthase, which catalyzes the synthesis of ACC from *S*-adenosyl Met, and ACC oxidase, which catalyzes the conversion of ACC to ethylene (Adams and Yang, 1979). Genes coding for ACC synthase have been cloned and sequenced from a number of species including tomato (*Lycopersicon esculentum* Mill.; Van Der Straeten et al., 1990), winter squash (*Cucurbita maxima* Duch; Nakajima et al., 1990), and zucchini (*Cucurbita pepo;* Sato and Theologis, 1989). For apple (*Malus domestica* Borkh.), a partial ACC synthase cDNA clone has been isolated (Dong et al., 1991), but to date a full-length clone has not been reported.

Here we report the isolation and characterization of a full-length cDNA clone (pAPAS) encoding apple ACC synthase. The pAPAS clone was isolated from a library constructed from poly(A^+) RNA extracted from ripe apple fruit (*M. domestica* Borkh. cv Golden Delicious) (Ross et al., 1992). This library was screened with pAAS2, a 1.6-kb partial cDNA clone encoding apple ACC synthase (Dong et al., 1991). The nucleic acid sequence of pAPAS is 2021 bp and contains an open reading frame encoding a 473-amino acid polypeptide (Table I). The cDNA sequence contains 126 bases at the 5′ end and 278 bases at the 3′ end, which are additional to that of pAAS2. The extra sequence at the 5′ end includes a putative translation start codon at position 100 and sequence encoding nine N-terminal amino acids not present in pAAS2. Relative to pAAS2 there are base substitutions in pAPAS at positions 129 (T → C), 135 (A → T), 141 (G → C), 142 (A → C), 203 (T → A), 400 (A → G), and 405 (T → C), resulting in amino acid changes at positions 141 (E → Q), 202 (L → H), and 400 (K → E). In addition, during sequencing of pAPAS we noticed and informed Dong et al. that they may have omitted five bases, corresponding to nucleotides 1424 to 1428 in the open reading frame of pAPAS in their published pAAS2 sequence (Dong et al., 1991). They have since confirmed this error and deposited a corrected sequence in the GenBank data base (accession No. U03294).

Table I. *Characteristics of pAPAS cDNA from apple fruit*

Organism:
Apple (*Malus domestica* Borkh. cv Golden Delicious).

Techniques:
Isolated from a cDNA library in pSPORT1 (Ross et al., 1992) using pAAS2 (Dong et al., 1991) as probe. Complete double-stranded cDNA sequence was obtained by sequencing of subclones and the use of synthetic oligonucleotide primers.

Method of Identification:
Sequence comparison showed high homology with other functionally identified ACC synthase cDNA clones. *Escherichia coli* transformed with pAPAS showed specific ACC synthase activity.

Features of mRNA:
Detected by northern analysis in ripening apple fruit. Two bands, 2.0 and 1.3 kb in size, hybridized to pAPAS probe. In ripening apple fruit, pAPAS complementary RNA was first detectable in fruit with an internal ethylene concentration of 0.4 μL L^{-1}, and steady-state levels of pAPAS complementary RNA increased with increasing fruit ethylene production.

Features of cDNA Clone:
The pAPAS clone contains 99 nucleotides of 5′ untranslated sequence, an open reading frame of 1419 nucleotides in length, and 503 nucleotides of 3′ untranslated sequence. A putative polyadenylation signal (AATTAA) is present at position 1806 and two seven-nucleotide (GTGTGTG) repeats are present at positions 1816 and 1837.

Features of the Deduced Protein:
The pAPAS open reading frame encodes a 473-amino acid deduced polypeptide with a predicted mass of 53.2 kD and a calculated pI of 7.0. The subcellular location has not been determined.

Chromosomal Localization:
Unknown; according to Southern data, gene copy number is low.

Northern analysis showed that the steady-state level of pAPAS complementary RNA in ripening apple fruit increases with ethylene production by the fruit. Two bands were observed, 2.0 and 1.3 kb in size, hybridizing to pAPAS probe. During ripening, pAPAS complementary RNA was first detectable in fruit with an internal ethylene concentration of 0.4 μL L^{-1}, compared with ACC oxidase mRNA, which was detectable in fruit at an earlier ripening stage (internal ethylene concentration $\leq$ 0.2 μL L^{-1}). This finding is in agreement with work on tomato, which showed that during ripening TOM13 mRNA, which en-

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codes tomato ACC oxidase (Hamilton et al., 1991), is detectable before ACC synthase mRNA (Oeller et al., 1991).

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