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Plant Gene Register

Nucleotide Sequence of a cDNA for 1-Aminocyclopropane-1-Carboxylate Synthase from Melon Fruits[1]

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Ethylene is a plant hormone that has an essential role in fruit ripening (Yang and Hoffman, 1984; Kende, 1993). ACC synthase (*S*-adenosyl-L-methionine methylethioadenosine-lyase, EC 4.4.1.14), which is encoded by a multigene family, plays a regulatory role in ethylene production. Several genes for ACC synthase have been isolated from tomato (Rottmann et al., 1991), mung bean (Botella et al., 1992, 1993), winter squash (Nakajima et al., 1990; Nakagawa et al., 1991), and *Arabidopsis* (Liang et al., 1992; Van Der Straeten et al., 1992). Two ACC synthase genes (LE-ACS2 and LE-ACS4, which are identified as a wounding and a ripening inducing gene, respectively) are expressed during ripening of tomato fruits (Olson et al., 1991; Rottmann et al., 1991). An antisense RNA experiment with LEACS2 reduced the levels of mRNAs for LEACS2 and LEACS4 in tomato fruits and caused retardation of initiation of ripening of tomato fruits (Oeller et al., 1991). These results showed that wound-induced ACC synthase also played an important role in the production of ethylene in tomato fruit during ripening.

We isolated a cDNA (pMEACS1, 2097 bp) for ACC synthase from wounded mesocarp tissue of melon fruits (*Cucumis melo* L. cv AMS) (Table I). The polypeptide derived from the cDNA in *Escherichia coli* had ACC synthase activity. Sequence analysis of this cDNA revealed the presence of an open reading frame of 493 amino acids. This polypeptide contained seven sequences that were conserved among other ACC synthases. pMEACS1 showed high homology at the amino acid and nucleotide levels to wound-induced ACC synthase from squash (Nakajima et al., 1990; Sato et al., 1991). RNA blot analysis showed that the level of mRNA for the gene increased in the mesocarp tissue of melon fruits after wounding and also during ripening. Since we could detect cDNA only for MEACS1 ACC synthase in a PCR experiment with the mRNA from mesocarp tissue of ripe melon fruits, MEACS1 should be the gene that is preferentially expressed during ripening of melon fruits. This result suggests that MEACS1, the wound-inducible ACC synthase, plays a major role in the vigorous increase in ethylene production in melon fruits during the climacteric rise of ethylene production.

Table I. *Characteristics of a cDNA coding for an ACC synthase of melon fruits*

Organism:
Cucumis melo L cv AMS.
Gene Product:
1-Aminocyclopropane-1-carboxylate synthase; *S*-adenosyl-L-methionine methylthioadenosine lyase; EC 4.4.1.14.
Source:
cDNA library in Bluescript SK(−) prepared from mRNA isolated from wounded slices (5-mm-thick slices that had been incubated for 9 h at 25°C) of mesocarp tissue of preclimacteric fruits.
Techniques:
About 1.1 kb of cDNA for ACC synthase was amplified from cDNA library by the PCR using mixed oligonucleotide primers. The upstream primer was CAAATGGGT(C/T)T-(GATC)GC(TA)GA(AG)AATCAGCT and the downstream primer was CAT(AG)TT(TG)GC(AG)AA(AG) CAAAC(AT)C-G(AG)AACCA(CA)CCTGG(CT)TC. cDNAs that contained the entire coding region were isolated from the cDNA library with the PCR-amplified cDNA labeled with biotin.
Method of Identification:
Polypeptides produced in *E. coli* had ACC synthase activity.
Structural Features of the cDNA Clones:
The clone was 2096 bp in length and possessed an open reading frame of 1479 bp, which encodes a protein of 493 amino acids. The open reading frame began with the ATG codon at position 103 and terminated at the codon TAA at position 1582. pMEACS1 shows 78.9% similarity at the nucleotide level and 84.6% similarity at the amino acid level with pCMW33, a wound-induced ACC synthase from winter squash (Nakajima et al., 1990). pI of the ACC synthase was calculated to be 6.41.
Subcellular Localization:
Not determined.
Antibody:
Not available.

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