Ethylene, a gaseous plant hormone, is involved in regulation of various physiological responses during plant growth and development. These include seed germination, abscission, fruit ripening, and plant senescence (Yang and Hoffman, 1984). Plants also produce high levels of ethylene when they are under environmental stresses or pathogen attacks. In ethylene biosynthesis, the precursor Met is converted to SAM via the catalysis of the enzyme SAM synthetase. SAM is further converted to ACC by ACC synthase (EC 4.4.1.14), which is the rate-limiting step of ethylene production. This reaction can be inhibited by exogenous application of aminoethoxyvinylglycine or aminooxyacetic acid, both of which are inhibitors of pyridoxal phosphate, which is required in ACC synthase activity. The last step of ethylene biosynthesis, ACC-ethylene, is catalyzed by ACC oxidase (ethylene-forming enzyme) (Yang and Hoffman, 1984).

ACC synthase has been purified to homogeneity from tomato fruits, and the corresponding genes have been isolated (Van Der Straeten et al., 1990), although the enzyme usually is present at low concentrations. To date, genes encoding ACC synthase have been cloned from several plant species including monocots and dicots (Theologis, 1992). In tomato, at least six different genes for ACC synthase have been identified, indicating that the enzyme is encoded by a multigene family (Rottmann et al., 1991). Two of the genes have been shown to be differentially expressed in ripening fruits after wounding. In transgenic experiments, tomato plants expressing antisense ACC synthase RNA displayed a great reduction of ethylene production, concomitant with a delay of the fruit-ripening process (Oeller et al., 1991). We have previously shown the regulatory role of ethylene in shoot morphogenesis of recalcitrant Brassica species in vitro (Chi et al., 1991; Pua, 1993; Pua and Chi, 1993) and have also cloned a cDNA encoding ACC oxidase from mustard (Pua et al., 1992). We report in this study a cDNA clone encoding ACC synthase, designated pMACC, isolated from mustard (Brassica juncea).

The cDNA contained one open reading frame encoding a cDNA clone encoding 1-Aminocyclopropane-1-Carboxylate Synthase in Mustard (Brassica juncea [L.] Czern & Coss)1

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Table 1. Characteristics of MACC cDNA from mustard

| Function: Encodes ACC synthase (EC 4.4.1.14). |
| Techniques: The cDNA library was constructed as previously described (Pua et al., 1992). For library screening, a 340-bp homologous probe was amplified from the cDNA library by the polymerase chain reaction using mixed oligonucleotide primers. The upstream primer was CTGGATCCGTCAGA(C/T)C(C/A/T/G)(T/C)T(A/G)CCCAC(C/A/T/G)AC and the downstream primer was CTCAAGCTTAC(A/C/T/G)A(A/C)(C/A/T/C)CC(A/G)AA/A(G)CT(C/T)GACAT, as designed by Kim et al. (1992). The clone was identified by Southern blot analysis and linearized, and the insert was restricted with exonuclease III to generate a nested set of deletions using the Erase-a-Base system (Promega Corp., Madison, WI). The nucleotide sequence of the insert was determined by the dideoxy chain termination method using Taq DNA polymerase (Promega Corp.). |
| Method of Identification: Sequence comparison showed high homology with the tomato ACC synthase gene LE-ACC2 (Rottmann et al., 1991). Both genes shared the overall identity of 64.1% in nucleotide sequence and 60.5% in deduced amino acid sequence. |

Feature of the cDNA: The clone was 1780 bp in length and possessed an open reading frame of 1560 bp, which encodes a protein of 497 amino acids. In transgenic experiments, tomato plants expressing antisense ACC synthase RNA displayed a great reduction of ethylene production, concomitant with a delay of the fruit-ripening process (Oeller et al., 1991). We have previously shown the regulatory role of ethylene in shoot morphogenesis of recalcitrant Brassica species in vitro (Chi et al., 1991; Pua, 1993; Pua and Chi, 1993) and have also cloned a cDNA encoding ACC oxidase from mustard (Pua et al., 1992). We report in this study a cDNA clone encoding ACC synthase, designated pMACC, isolated from mustard (Brassica juncea).

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Abbreviation: SAM, S-adenosylmethionine.
protein of 497 amino acids, with an estimated molecular mass of 55.5 kD (Table I). Both Leu (9.05%) and Ala (7.85%), as in ACC oxidase (Pua et al., 1992), were the most abundant amino acids in the MACC protein. Comparison of a highly conserved region (amino acid positions 211–311) of the MACC cDNA with ACC synthase genes of other plant species showed the highest homology, 69%, with AT-ACC1 of Arabidopsis thaliana (Van Der Straeten et al., 1992), 65% with LE-ACC2 of tomato (Rottmann et al., 1991), and 52% with OS-ACC1 of rice (Theologis, 1992). The degree of homology may be related to the phylogenetic relationship between mustard and these plant species, among which A. thaliana is closer to mustard. Nevertheless, MACC contained domains highly conserved in ACC synthase genes, including the active site of pyridoxal phosphate attachment, of both monocots and dicots (Theologis, 1992), indicating the common evolutionary origin of the genes.

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