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Plant Gene Register

A cDNA Sequence Encoding 1-Aminocyclopropane-1-Carboxylate Oxidase from Pea[1]

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The role of ethylene in keeping the apical hook of many etiolated dicotyledonous seedlings tightly closed is well established (Abeles, 1973). The hook results from an asymmetry in growth between the upper and the lower sides of the stem. There is evidence that this differential growth may be the result of asymmetric distribution of ethylene biosynthesis (Schierle and Schwark, 1988). To study the distribution and regulation of the ethylene biosynthetic enzymes in apical hooks of pea (*Pisum sativum* L. cv Alaska), it is necessary to isolate clones encoding ACC synthase and the ethylene-forming enzyme, ACC oxidase.

From a comparison of amino acid sequences of pTOM13 from tomato (Holdsworth et al., 1987) and an ACC oxidase homolog from avocado (McGarvey et al., 1990), we selected two conserved amino acid regions (FGTKVSN and PKEPRFE) for synthesis of nondegenerate oligonucleotide primers corresponding to the respective nucleotide sequence of pTOM13. PCR-based amplification was carried out using these oligonucleotides as primers and cDNA from a pea library as template. The PCR product was sequenced to confirm its homology to pTOM13 and used to screen a cDNA library in Lambda Zap II (Stratagene, La Jolla, CA) made from poly(A^+) RNA from apical hooks of etiolated peas treated with 10^{-4} M IAA for 4 h. Eight clones were isolated and found to be identical by sequencing. The insert of the longest full-length cDNA, pPE8, was 1122 bp long and contained an open reading frame encoding 317 amino acids. The nucleotide and deduced amino acid sequences were highly homologous to those of tomato ACC oxidase cDNAs (Hamilton et al., 1991; Spanu et al., 1991) and to other related sequences (McGarvey et al., 1990; Wang and Woodson, 1991; Dong et al., 1992). The insert from pPE8 was subcloned into the *Eco*RI site of the yeast expression vector pYES2.0 (Invitrogen, San Diego, CA) and used to transform *Saccharomyces cerevisiae* strain F808. Using previously described methods (Peck et al., 1992), the transgenic protein was shown to have ACC oxidase activity and to be recognized on immunoblots by antibodies raised against tomato ACC oxidase.

Table I. *Characterization of PE8 cDNA from apical hooks of etiolated peas*

Organism:
Pea (*Pisum sativum* L. cv Alaska).

Techniques:
PCR-based amplification to generate a fragment (PE1) of ACC oxidase, which was used to isolate a full-length clone from a Lambda Zap II cDNA library (Stratagene, La Jolla, CA); restriction fragment subclones and synthetic oligonucleotide primers were used to completely sequence both strands.

Method of Identification:
Sequence comparison showed high homology with other functionally identified ACC oxidase cDNA clones. Yeast transformed with PE8 subcloned into the yeast expression vector pYES2.0 showed specific ACC oxidase activity. Antibodies raised to tomato ACC oxidase recognized the pea protein expressed in yeast by immunoblotting.

Features of the Deduced Protein:
Open-reading frame 317 amino acids: M_r 36,053; modified Chou-Fosman analysis indicates an amphipathic helix from Val^{113} to Glu^{134} with the helix possibly continuing to Lys^{145}. Beginning with Phe^{117}, this helix contains a potential Leu zipper that may be involved in protein-protein interactions.

Antibodies:
None available.

Computer analysis of the protein sequence using modified Chou-Fosman and Amphi programs (available from A.R. Crofts, University of Illinois, Urbana, IL) predicted an amphipathic helix from Val^{113} to Glu^{134} and possibly extending to Lys^{145}, although this portion of the analysis was inconclusive. Beginning with Phe^{117}, which is known to substitute for Leu residues in Leu zippers without a loss of function (Gentz et al., 1989), there exists a Leu repeat every seven amino acids (^{117}FALKLEELAEELLDLLCENLGL^{138}), a characteristic of a Leu zipper. This motif is conserved in all known ACC oxidase and putative ACC oxidase sequences and in the corresponding sequence of E8 protein, which is the product of an ethylene-regulated gene in tomatoes (Deikman and Fischer, 1988). This structure may be involved in protein-protein interactions related to the function of the protein(s).

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Abbreviation: PCR, polymerase chain reaction.

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