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Plant Gene Register

Nucleotide Sequence of a cDNA Encoding the Ethylene-Forming Enzyme from Petunia Corollas[1]

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An increase in the production of ethylene is associated with specific tissues during development and in response to abiotic and biotic stresses (10). In most tissues, the increase in ethylene synthesis is the result of increased ACC[2] synthase activity, which catalyzes the conversion of AdoMet to ACC. An exception to this, however, is tissues that exhibit an increase in ethylene production associated with senescence, including ripening fruit and senescing flowers. In these tissues, ethylene production is regulated by both ACC synthase and EFE, which oxidizes ACC to ethylene (10). The role of EFE in the regulation of ethylene biosynthesis in these tissues has led to considerable interest in the molecular mechanisms underlying EFE expression. Early progress on the purification and characterization of EFE was slow due to the lack of a suitable cell-free enzyme assay. A significant advancement was made when the expression of an antisense fruit ripening-related mRNA in transgenic tomatoes was found to inhibit ethylene production by blocking the conversion of ACC to ethylene (3). This led to the speculation that the ripening-related mRNA represented by this cDNA encoded the elusive EFE. It was noted by Hamilton et al. (3) that the deduced protein of pTOM13 shared significant homology with a flavanone-3-hydroxylase from *Antirrhinum majus*. This led Ververidis and John (8) to assay EFE in vitro as a hydroxylase in the presence of Fe^{2+} and ascorbate. Indeed, these researchers were able to establish authentic EFE activity in soluble plant extracts by this assay. Recently, the identity of pTOM13 as representative of a tomato ripening-related EFE was confirmed by expression of homologous transcripts and recovery of enzyme activity in *Xenopus* oocytes (7) and yeast (2).

In the flowers of petunia, pollination induces senescence of the corolla through the increased synthesis and action of ethylene. We are interested in the regulation of ethylene biosynthesis during flower senescence and report here the nucleotide sequence of a cDNA (pPHEFE) from senescing petunia corollas that represents an EFE mRNA. The sequence of pPHEFE is 1238 bp and contains an open reading frame of 319 amino acids (Fig. 1). pPHEFE shares greater than 77% nucleotide sequence homology with a fruit ripening-related EFE cDNA clone from tomato (4). The predicted protein of pPHEFE (Table I) shares extensive homology with other known EFEs (1, 5, 6, 9) and is greater than 90% identical to the tomato protein, which has been proven to encode EFE (2).

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene-forming enzyme.

Figure 1. Nucleotide and deduced amino acid sequence of petunia pPHEFE.

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   1  GTTATTCACACACTATATACTCTAAAAACACATTCTTCATTCTGCAAGAAAGAAAATGGA
                                                              M  E    2
  61  GAACTTCCCAATTATCAGCTTGGACAAAGTGAATGGTGTTGAAAGAGCTGCCACTATGGA
        N  F  P  I  I  S  L  D  K  V  N  G  V  E  R  A  A  T  M  E   22
 121  AATGATTAAGGATGCATGTGAAAACTGGGGCTTCTTTGAGTTGGTGAACCATGGAATCCC
        M  I  K  D  A  C  E  N  W  G  F  F  E  L  V  N  H  G  I  P   42
 181  ACGTGAAGTAATGGACACTGTGGAGAAGATGACAAAGGGTCATTACAAGAAGTGCATGGA
        R  E  V  M  D  T  V  E  K  M  T  K  G  H  Y  K  K  C  M  E   62
 241  ACAAAGGTTCAAGGAATTGGTTGCCAGTAAAGCTCTTGAAGGTGTTCAAGCTGAGGTTAC
        Q  R  F  K  E  L  V  A  S  K  A  L  E  G  V  Q  A  E  V  T   82
 301  TGATATGGATTGGGAAAGCACCTTTTTCTTGAAACATCTCCCCATTTCTAACATTTCTGA
        D  M  D  W  E  S  T  F  F  L  K  H  L  P  I  S  N  I  S  E  102
 361  AGTCCCTGATCTTGATGAAGAATACAGGGAAGTTATGAGAGATTTTGCTAAAAGGTTAGA
        V  P  D  L  D  E  E  Y  R  E  V  M  R  D  F  A  K  R  L  E  122
 421  GAAGCTGGCAGAGGAGCTTTTGGACTTGCTGTGTGAAAATCTTGGCCTGGAAAAAGGTTA
        K  L  A  E  E  L  L  D  L  L  C  E  N  L  G  L  E  K  G  Y  142
 481  CCTTAAAAATGCCTTTTATGGATCGAAAGGTCCAAACTTTGGGACTAAAGTGAGCAACTA
        L  K  N  A  F  Y  G  S  K  G  P  N  F  G  T  K  V  S  N  Y  162
 541  TCCACCATGCCCAAAACCAGATTTAATCAAAGGACTACGTGCCCACACAGACGCTGGTGG
        P  P  C  P  K  P  D  L  I  K  G  L  R  A  H  T  D  A  G  G  182
 601  AATAATCCTTCTGTTCCAAGATGATAAAGTAAGTGGCCTACAACTCCTCAAAGACGGCCA
        I  I  L  L  F  Q  D  D  K  V  S  G  L  Q  L  L  K  D  G  Q  202
 661  ATGGATCGATGTTCCTCCCATGCGCCATTCCATTGTGGTTAACCTCGGTGACCAACTCGA
        W  I  D  V  P  P  M  R  H  S  I  V  V  N  L  G  D  Q  L  E  222
 721  GGTGATCACTAATGGAAAATACAAGAGTGTGATGCACAGAGTGATAGCCCAAAAAGACGG
        V  I  T  N  G  K  Y  K  S  V  M  H  R  V  I  A  Q  K  D  G  242
 781  GGCTCGAATGTCATTAGCTTCCTTTTACAATCCAGGAAGTGATGCAGTGATCTATCCAGC
        A  R  M  S  L  A  S  F  Y  N  P  G  S  D  A  V  I  Y  P  A  262
 841  ACCAGCTCTTGTTGAGAAAGAAGCAGAGGAAAACAAACAAGTCTACCCAAAATTTGTATT
        P  A  L  V  E  K  E  A  E  E  N  K  Q  V  Y  P  K  F  V  F  282
 901  TGATGATTACATGAAGTTATATGCTGGACTCAAGTTTCAAGCTAAGGAGCCAAGATTTGA
        D  D  Y  M  K  L  Y  A  G  L  K  F  Q  A  K  E  P  R  F  E  302
 961  AGCAATGAAAGCCATGGAAACTGATGTCAAGATGGATCCAATTGCCACTGTCTAATAGAT
        A  M  K  A  M  E  T  D  V  K  M  D  P  I  A  T  V           319
1021  GGAAGTTGGATAAAAATGATTTGTTATTAAAAGTAGTACAAGACCTGTGACATATATTAT
1081  ATGTTTCTTTAGTATAGTGTGATCAATCTATTTACAAGAGGGGTGTGTCCCTACTATATA
1141  TGTAGTTTGTCCCTACTACATATGTAGTTAAGTGGCCTAAAGTTTGTATCTTATACTAAA
1201  CTGATAAAGCTTCTTTTTACACAAATATTAATATACCC
```

Table 1. *Characteristics of an EFE cDNA (pPHEFE) from Petunia*

Organism

Petunia hybrida L. 'Pink Flash'.

Function:

Encodes ACC oxidase (EFE), which catalyzes the conversion of ACC to ethylene.

Clone Type; Designation:

cDNA, full-length; pPHEFE

Source:

cDNA library in λZAP vector constructed using poly(A)$^+$RNA isolated from senescing petunia corollas 4 to 5 d after pollination.

Method of Identification:

Library was screened with the cDNA pSR120 (9), which represents EFE mRNA from senescing carnation flower petals.

Sequencing Strategy:

Plasmid sequencing; restriction fragment and exonuclease III deletion subclones used to completely sequence both strands.

Expression Characteristics:

Transcript of approximately 1300 nucleotides; increase in mRNA abundance in senescing corolla tissue and pollinated pistil concomitant with increased ethylene production; increase in mRNA detected in corollas following ethylene exposure.

Features of cDNA Structure:

Nucleotide sequence is 77% identical to the tomato EFE cDNA pTOM13 (4).

Structural Features of Deduced Protein:

319 amino acids (Mr 36,186); pI of 5.17; 92%, 78%, 76%, and 75% identical to predicted EFE proteins from tomato (4), apple (1), avocado (5, 6), and carnation (9), respectively.

Antibodies:

None available.

Subcellular Location:

Not determined.

GenBank Accession No.:

M90294