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Plant Gene Register

Nucleotide Sequence of a cDNA Encoding Malic Enzyme from Poplar[1]

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NADP-dependent ME[3] (EC 1.1.1.40) catalyzes the oxidative decarboxylation of malate to pyruvate-generating reducing equivalents (NADPH + H^+) and CO_2. In plants, both cytosolic and chloroplastic NADP-ME have been found (6).

A leaf cDNA library from a poplar hybrid (*Populus deltoides* × *P. trichocarpa* clone 064) constructed in the vector pUC18 was screened with an NADP-ME cDNA clone from *Phaseolus vulgaris* (7, 8) (Table I). Figure 1 shows the nucleotide sequence of the 2071-bp cDNA insert and the derived amino acid sequence. The cDNA contains one continuous open reading frame corresponding to a protein of 589 amino acid residues (calculated M_r 64,900). The translation initiation codon is preceded by an in-frame stop codon. The polypeptide is 79 and 73% homologous with the NADP-ME from bean (8) and maize (4), respectively. Neither the poplar nor the bean NADP-ME polypeptides contain an apparent transit sequence for translocation to the chloroplasts, suggesting that they are located in the cytosol. Northern hybridizations revealed that the amounts of steady-state NADP-ME mRNA is twofold higher in leaves and stems of *in vitro* grown poplar plants as compared to the levels found in roots (data not shown).

Table I. *Characteristics of cDNA pPOPCAD1 from Poplar*

Organism: *Populus deltoides* × *Populus trichocarpa*, clone 064 (Afocel, France).
Location on chromosome: Unknown.
Gene product; pathway: NADP-dependent ME (EC 1.1.1.40); Calvin cycle and other metabolic pathways (1, 4).
Techniques: Plasmid sequencing (5); RNA preparation (2); northern analysis (3).
Method of identification: Sequence comparison with a chloroplastic NADP-dependent ME from maize (73% homology) and a cytosolic NADP-dependent ME from bean (79% homology) (4).
Expression characteristics: Transcript of about 2200 nucleotides; transcript termini not determined.
Regulation: Unknown.
Features of cDNA structure: Putative polyadenylation signal (AATAAA at position 2008).
Codon usage: No significant differences in codon usage.
(G + C) content: 44.5%.
Structural features of protein: ORF 589 amino acids; M_r 64,900; protein expression not tested; putative charge at pH 7 = 6.36; proposed NADP-binding site VYTPTVG (positions 138–144); proposed NAD-binding site ILGLGDLG (positions 193–200); the proposed NAD- and NADP-binding sites are each followed by a cysteine (6, and references therein).
Antibodies: None available.
Subcellular location: Not tested, most probably cytosolic.
EMBL accession No.: X56233.

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[3] Abbreviation: ME, malic enzyme.

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   1                                                AGCAAACCACTACTCCAGAACTTGTTATTTAATCTCTATATATTTTGCTGTTT
  54  CTCCCTTTGATTTGGAAAGAAAGAAAGGTCAGGTTTAGGTTTCGGTTCGGTTTAGTTTGATATTCGAAGATCAGCAGAGAGAGATTAAGTCAACTGAGA
 153  ATGGAGAGCACGCTGAAGGAGATGAGAGACGGAGCTTCGGTGCTCGACATGGACCCCAAATCCACTGTCGGTGGTGGTGTTGAGGACGTTTATGGCGAG
       M  E  S  T  L  K  E  M  R  D  G  A  S  V  L  D  M  D  P  K  S  T  V  G  G  G  V  E  D  V  Y  G  E    33
 252  GATCGTGCCACTGAGGACCAGCTTGTTACTCCCTGGACCATCTCCGTTGCCAGTGGATATACTTTGTTGAGGGATCCACATCACAACAAAGGGCTTGCT
       D  R  A  T  E  D  Q  L  V  T  P  W  T  I  S  V  A  S  G  Y  T  L  L  R  D  P  H  H  N  K  G  L  A    66
 351  TTCACTGAGAAAGAAAGAGATGCACACTACCTGCGTGGTCTTCTTCCTCCAACAACTATCTCTCAACAGCTTCAGGAAAAGAAATTGATGAACACCATT
       F  T  E  K  E  R  D  A  H  Y  L  R  G  L  L  P  P  T  T  I  S  Q  Q  L  Q  E  K  K  L  M  N  T  I    99
 450  AGACAATATCAACTTCCTCTTCAAAAATATACGGCCATGATGGAACTTGAGGAAAGGAATGAAAGGTTGTTTTATAAGCTTTTGATCGATAATGTTGAG
       R  Q  Y  Q  L  P  L  Q  K  Y  T  A  M  M  E  L  E  E  R  N  E  R  L  F  Y  K  L  L  I  D  N  V  E   132
 549  GAATTGCTTCCGGTTGTTTATACACCGACGGTTGGGGAAGCTTGCCAGAAGTATGGAAGTATTTTCAAGCGTCCTCAGGGTCTATATATAAGTTTAAAA
       E  L  L  P  V  V  Y  T  P  T  V  G  E  A  C  Q  K  Y  G  S  I  F  K  R  P  Q  G  L  Y  I  S  L  K   165
 648  GAGAAGGGGAAAGTTCTTGATGTGCTGAAAAACTGGCCTCAGAAGAGTATTCAAGTTATTGTTGTTACTGATGGTGAAAGAATTTTGGGACTTGGGGAT
       E  K  G  K  V  L  D  V  L  K  N  W  P  Q  K  S  I  Q  V  I  V  V  T  D  G  E  R  I  L  G  L  G  D   198
 747  CTTGGCTGTCAGGGAATAGGCATTCCTGTTGGGAAGTTGTCTCTTTACACTGCACTTGGAGGAGTTCGTCCTTCAGCTTGTTTGCCCGTAACCATTGAT
       L  G  C  Q  G  I  G  I  P  V  G  K  L  S  L  Y  T  A  L  G  G  V  R  P  S  A  C  L  P  V  T  I  D   231
 846  GTGGGTACGAACAACGAGCAATTGCTGAAGGATGAATTCTACATTGGACTGAGACAAAGGAGGGCAACTGGCCAGGAATACTCTGAACTTCTCCATGAG
       V  G  T  N  N  E  Q  L  L  K  D  E  F  Y  I  G  L  R  Q  R  R  A  T  G  Q  E  Y  S  E  L  L  H  E   264
 945  TTCATGACTGCTGTAAAACAAAATTATGGGGAGAAAGTTCTGATACAGTTTGAAGATTTTGCTAACCACAATGCTTTTGATCTGCTTGCAAAGTATGGC
       F  M  T  A  V  K  Q  N  Y  G  E  K  V  L  I  Q  F  E  D  F  A  N  H  N  A  F  D  L  L  A  K  Y  G   297
1044  ACAACTCATTTAGTCTTCAATGATGATATACAGGGGACGGCTGCTGTTGTTCTTGCAGGGCTTATTTCAGCACTGAAGTTGCTAGGTGGTTCCCTGGCT
       T  T  H  L  V  F  N  D  D  I  Q  G  T  A  A  V  V  L  A  G  L  I  S  A  L  K  L  I  G  G  S  L  A   330
1143  GATCACACTTTCTTGTTCCTTGGTGCTGGTGAAGCTGGAACTGGTATAGCAGAGCTTATAGCTCTTGAGATGTCAAGACGGTCAAAAACTCCTTTGGAA
       D  H  T  F  L  F  L  G  A  G  E  A  G  T  G  I  A  E  L  I  A  L  E  M  S  R  R  S  K  T  P  L  E   363
1242  GAGACTCGCAAGAAGATTTGGCTGACAGATTCAAAGGGTTTGATTGTTAGTTCTCGCAAGGAGTCATTACAACACTTCAAGAAACCATGGGCTCATGAA
       E  T  R  K  K  I  W  L  T  D  S  K  G  L  I  V  S  S  R  K  E  S  L  Q  H  F  K  K  P  W  A  H  E   396
1341  CATGAACCCGTTAAAGGACTCCTAGAAGTTGTCAAGGCAATCAAACCAATAGTCTTGATTGGAACATCCGGAGTGGGAAAAACGTTTACTAAGGAAGTG
       H  E  P  V  K  G  L  L  E  V  V  K  A  I  K  P  L  V  L  I  G  T  S  G  V  G  K  T  F  T  K  E  V   429
1440  ATTGAGGCCATGGCTTCTTTCAATGAGAAACCTCTAATTTTGGCTCTCTCCAACCCAACCTCACAATCTGAATGTACAGCTCAAGAAGCCTACACTTGG
       I  E  A  M  A  S  F  N  E  K  P  L  I  L  A  L  S  N  P  T  S  Q  S  E  C  T  A  E  E  A  Y  T  W   462
1539  ACTAAGGGCAAAGCAATTTTTGCTAGTGGAAGTCCATTTGATCCTGTTGAGTACGAGGGAAAAGTTTTTGTTCCTGGCCAGTCCAACAATGCTTACATT
       G  K  G  K  A  I  F  A  S  G  S  P  F  D  P  V  E  Y  E  G  K  V  F  V  P  G  Q  S  N  N  A  Y  I   495
1638  TTCCCTGGCCTGGGTTTGGTCATCTCTGGAGCTATTCGTGTTCATGATGATATGCTTCTGGCAGCCGCGGAAGCCTTGGCTGGGCAAATTAAAGAAGAG
       F  P  G  L  G  L  V  I  S  G  A  I  R  V  H  D  D  M  L  L  A  A  A  E  A  L  A  G  Q  I  K  E  E   528
1737  TACTTGGCAAAGGGGCTGATTTACCCACCTCTATCTAACATCAGAAAAATCTCAGTCCAAATTGCTGCTAACGTAGCTGCCAAGGCATATGAACTTGGC
       Y  L  A  K  G  L  I  Y  P  P  L  S  N  I  R  K  I  S  V  Q  I  A  A  N  V  A  A  K  A  Y  E  L  G   561
1836  CTGGCTACACGTCTCCCCCGTCCAGAAAACCTTGTGAAGCACGCAGAGAGTTGCATGTACAGTCCCGCCTATAGATATTACCGGTGAATTGTCTGTACT
       L  A  T  R  L  P  R  P  E  N  L  V  K  H  A  E  S  C  M  Y  S  P  A  Y  R  Y  Y  R                  589
1935  GTGCTTGTGCATTTATGCTTTCTTTTGGTGTGAAGCCGCGTGCTGTCTTGAGAGCAACCGCTCTAATAAGCAAAATAAATTGTCTGGGCTTGTAATATC
2034  AGTACCTTGTTAAACTAGTAAATGGAGCTACATTTCTA
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Figure 1. Nucleotide sequence of the 2071-bp cDNA insert and the derived amino acid sequence of pPOPCAD1 from poplar.