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Plant Gene Register

Ascorbate Peroxidase cDNA from Maize[1]

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APx (EC 1.11.1.11) is a heme-containing, nonglycosylated enzyme that destroys harmful hydrogen peroxide via the ascorbate-glutathione pathway in plants. This pathway provides protection against oxidative stress by a series of coupled redox reactions, particularly in photosynthetic tissues (Foyer and Halliwell, 1976). Three types of APx are known in plants: a cytosolic APx, a chloroplastic stromal APx, and a thylakoid membrane-bound APx. They differ in mol wt, substrate specificity, pH optimum, and stability (for a review, see Creissen et al., 1994). The APx protein has been purified from pea, spinach, tea, and maize (*Zea mays*) (Chen and Asada, 1989; Mittler and Zilinskas, 1991a; Tanaka et al., 1991; Koshiba, 1993). Chloroplastic APx isoforms have been well characterized as scavengers of photoproduced hydrogen peroxide in the chloroplasts. The role of cytosolic APx in the cell still remains to be clarified. cDNA and genomic clones encoding cytosolic APx have been isolated from soybean root nodules (Chatfield and Dalton, 1993), pea (Mittler and Zilinskas, 1991b, 1992), and *Arabidopsis thaliana* (Kubo et al., 1992; S. Kushnir, H. Willekens, G. Bauw, M. Van Montagu, and D. Inzé, unpublished data). In maize, the N-terminal amino acid sequence of a purified APx showed high similarity to pea, spinach, and *Arabidopsis* cytosolic APx but not to chloroplastic APx from tea (Koshiba, 1993).

We report the isolation and sequencing of a maize cDNA clone coding for a cytosolic APx (Table I). A maize seedling λZAP library was screened with an *Arabidopsis* APx probe. Putative APx cDNA clones were isolated at a frequency of 1:40,000. A clone with an insert of 1.05 kb was purified until homogeneity and sequenced.

Sequence analysis of both strands resulted in a 1042-bp nucleotide sequence with a 3′ poly(A) tail and an open reading frame encoding 250 amino acids. Northern analysis showed an *apx* transcript of approximately 1 kb in 14-d-old seedlings, indicating that the isolated APx cDNA is full length. The coding region of the maize cDNA showed 73% similarity with the APx cDNAs isolated from pea and *A. thaliana* (Mittler and Zilinskas, 1991b; Kubo et al., 1992). A putative polyadenylation signal (ATAATA) was present 146 bp downstream from the stop codon (Joshi, 1987).

Table I. *Characteristics of a maize apx cDNA from maize*

Organism:
Maize (*Zea mays* L., line B73).
Clone, Source, Method of Identification:
pMAPX1, isolated from a cDNA λZAP expression library (Stratagene), prepared from leaf mRNA purified from 2-week-old, greenhouse-grown B73 seedlings, by probing with an *A. thaliana apx* cDNA clone (S. Kushnir, personal communication).
Features of the Sequence:
A total of 1042 bp, including a 71-bp leader and a 217-bp tailer sequence.
Gene Copy Number:
Two or three copies, as determined by Southern analysis (data not shown).
Structural Features of Deduced Protein:
250 amino acids (calculated pI, 5.25; calculated molecular mass, 27.3 kD).

The deduced APx polypeptide from maize showed 83% amino acid identity with the APx polypeptides from pea and *Arabidopsis*. The amino acid sequence from the N-terminal residue to the 30th residue from a previously reported maize APx (Koshiba, 1993) is clearly different from the maize APx reported here. The presence of at least two APx isozymes in maize was confirmed by Southern analysis of genomic DNA, which shows the presence of two or three *apx* gene copies in maize. A conserved amino acid sequence motif that is part of the active center in the Cyt *c* peroxidase is represented in the maize APx protein as His^{42}, His^{163}, and Arg^{38}.

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Abbreviation: APx, ascorbate peroxidase(s).

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