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SHORT COMMUNICATION

Cloning and characterization of a root sunflower peroxidase gene putatively involved in cell elongation

Maria Carmen Parra-Lobato, Maria Carmen Alvarez-Tinaut, Maria Carmen Gomez-Jimenez*

[a]*Department of Plant Physiology, Faculty of Science, University of Extremadura, 06071-Badajoz, Spain*

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Summary

A cDNA-encoding a peroxidase (*Helianthus annuus* POX (*HaPOX*)*1*) was isolated and characterized from the roots of sunflower seedlings. This gene exhibited homology with other peroxidases from several higher-plant species, and its expression in the root growth was particularly abundant during cell expansion. To elucidate the precise functions of HaPOX1 in sunflower root, we examined its expression pattern in response to several plant growth regulators. Expression of *HaPOX1* is down-regulated by abscisic acid (ABA), whereas indole-3-acetic acid (IAA) induced its expression. These results suggest that *HaPOX1* expression is differentially regulated by phytohormonal components of signaling cascades. Since IAA appears to participate in the regulation of *HaPOX1* expression, we postulate that the peroxidase encoded by *HaPOX1* may be involved in the reactions that promote cell elongation during the early stage of root growth.

Introduction

Class III plant heme peroxidases (POXs, EC 1.11.1.7) belong to a large family of plant secretory enzymes catalyzing oxidoreduction between a variety of phenolic substrates and hydrogen peroxide (Welinder, 1992). The large number of isoenzymes and the diversity of the processes catalyzed by POXs (Østergaard et al., 2000) involve them in a broad array of physiological and

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; HaPOX, *Helianthus annuus* POX; IAA, indole-3-acetic acid; JA, jasmonic acid; KN, Kinetin; POX, peroxidase; SA, salicylic acid

*Corresponding author. Tel.: +34 924 289359; fax: +34 924 289404.
E-mail address: mcgomez@unex.es (M.C. Gomez-Jimenez).

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developmental processes throughout the plant life cycle (Passardi et al., 2004). Expression of plant POX genes is complicated since they are regulated at different times and places by various kinds of biotic and abiotic stressors (Yoshida et al., 2003). Surprisingly, little is known about the precise functions of many POXs in plants. It has long been widely assumed for a long time that POXs are involved in growth inhibition (McAdam et al., 1992) but there are now indications that they could also promote growth (Chen and Schopfer, 1999; Dunand et al., 2002; Kawaoka et al., 2003; Passardi et al., 2006). The biochemical processes involved in cell wall loosening during extension growth are only partially known. Changes in internal cell wall structure can also be achieved by POXs through their peroxidative or hydroxylic cycles (Passardi et al., 2004).

To gather further information as to whether POX is associated with the growth of sunflower roots, in the present study, we isolated and characterized a cDNA encoding for one POX from sunflower roots. The expression of POX-mRNA was investigated during the early stage of root growth.

Materials and methods

Sunflower (*Helianthus annuus* L. cv dwarf) seeds were germinated in plastic trays (50 seeds and 175 mL distilled water) in darkness at 27 °C and 70% relative humidity. The roots were aseptically excised and immediately used or stored at −80 °C. Two-day-old seedlings were then treated with distilled water (control), abscisic acid (ABA), indole-3-acetic acid (IAA), jasmonic acid (JA), salicylic acid (SA), kinetin (KN) and 1-aminocyclopropane-1-carboxylic acid (ACC) for 24 h under light, at concentrations as described in the legend of Figure 2. Total RNA was extracted from roots of sunflower seedlings grown 72 h after sowing at 27 °C in water (darkness), using the RNeasy Plant kit according to the manufacture's instructions (Quiagen). Subsequently, first-strand cDNA was synthesized using the oligonucleotide 45628 [5′-CCGAATTCAATACGACTCACTATAGCG(T)15-3′] and MMuLV reverse transcriptase (Boehringer). The first PCR reaction was performed using the degenerate primers p1 [5′-CG(ATGC)TT(AG)CA(CT)TT(CT)CA(CT)-GA(CT)TG(CT)TT-3′] and p2 [5′-A(AG)TT(ATGC)CCCAT-(CT)TT(ATGC)A(CT)CAT-3′], homologous to highly conserved regions of the other POX previously isolated from higher plants. To obtain the 5′-cDNA sequence, RACE-PCR was performed using a kit from Gibco and poly(A)$^+$-RNA (10 μg). Poly(A)$^+$-RNA was purified from total RNA (roots of sunflower seedlings grown 72 h, using the RNeasy Plant kit) using PolyAtract System 1000 (Promega, Madison, WI, USA). The primer p3 [5′-CTCTGAGTTGGGTCCATCTAGTA-GAA-3′] was used for first-strand cDNA synthesis. A nested primer p4 [5′-AAGCAGTCGTGGAAGTGCAACCG-3′] together with the RACE anchor primer (Gibco) was used for amplification. After 30 cycles of PCR, a fragment of 180 bp was obtained and sequenced. The primers p5 [5′-CTGTCGTACGATTTCTACCA-3′] and p6 [5′-ATTGCCCATCTT-GACCATGG-3′] were used to obtain the PCR product of 831 bp (*Helianthus annuus* POX (*HaPOX*)*1*), which was cloned into plasmid pGEM-T (Promega) and sequenced in both directions. Total RNA (10 μg) was isolated from sunflower roots (using the RNeasy Plant kit, Quiagen) and subjected to RNA gel-blot analysis, which was performed as described previously (Perin et al., 2002) with ^{32}P-labeled *HaPOX1* PCR product (831 bp) as a probe.

Results and discussion

The present work reports on the cloning and characterization of one member of the sunflower POX gene family. A sense primer was designed based on the amino acid sequence and was used for RT-PCR, and the cDNA sequence was determined (Figure 1A; accession no. DQ889869 designated as *HaPOX1*). The *HaPOX1* clone predicted a 277-amino acid sequence containing a signal peptide at the N terminus (Figure 1A). A signal peptide sequence deduced from the cDNA sequence supports the conclusion that the protein is destined for localization in the cell wall. The primary structure of HaPOX1 shows a high number of tyrosine residues (Figure 1A), which have been associated with POX–lignin cross-linking, assumingly occurring in the plant cell wall (McDougal, 2001). HaPOX1 also contained the active site residues of the plant POX superfamily (Welinder et al., 2002), shown in bold in Figure 1A.

HaPOX1 showed moderate similarity with many homologues of plant POX (Figure 1B). A homology search of the PeroxiBase database (http://peroxidase.isb-sib.ch/; Bakalovic et al., 2006) indicated that HaPOX1 has the highest similarity of 68% to the amino acid sequence encoded by a putative POX gene of *Lactuca sativa*, LsPrx42. The deduced amino acid sequence of *HaPOX1* was 67%, 51% and 45% identical to that encoded by the cDNAs for POX obtained from *Lactuca perennis* (LperPrx41), *Arabidopsis thaliana* POX Atatp14a (X98803; The EST clone P60; Tognolli et al., 2002) and *Beta vulgaris* cationic POX (AM265608), respectively. The P60 transcript was detected in the roots only, but the function of this EST clone is unknown (Tognolli et al., 2002). Homologues with other dicots and monocots showed fewer similarities (35–45%). The phylogenetic analysis was performed by comparing the conserved domains of POX sequences from several species (Figure 1B).

To examine changes in the level of the *HaPOX1* mRNA during root growth, we isolated total RNA