Nucleotide Sequence of a Cationic Peroxidase Gene from the Tropical Forage Legume *Stylosanthes humilis*

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Peroxidases (EC 1.11.1.7; donor: hydrogen-peroxide oxidoreductase) have been implicated in numerous physiological processes of potential importance in plant-pathogen interactions, including lignification (Walter, 1992), cross-linking of cell-wall components (Bradley et al., 1992), wound healing (Sherf et al., 1993), auxin oxidation (Grambow and Langenbeck-Schwich, 1979), and systemic acquired resistance responses (Irving and Kuc, 1990). A complete description of peroxidase activity in vivo is not available because multiple isoenzymes exist with large numbers of potential substrates. A cDNA (*Shpx6*, EMBL accession No. L36110) encoding a putative cationic peroxidase was isolated from *Stylosanthes humilis*. Transcripts hybridizing to this cDNA were shown to be expressed in young leaves and roots of mature plants and were strongly induced in young leaves as a response to wounding and infection with the fungal phytopathogen *Colletotrichum gloeosporioides*, which causes anthracnose disease in *S. humilis* (Harrison et al., 1994). Southern hybridization analysis with genomic DNA of *S. humilis* indicated that there are probably two closely related copies of this cationic peroxidase gene (Curtis et al., 1995).

We report the isolation of one of the cationic peroxidase genes from *S. humilis*. A single cationic peroxidase gene with 83% nucleotide sequence homology in the coding region to the cDNA *Shpx6* was isolated from a genomic DNA library of *S. humilis* constructed in the λ phage EMBL3. The gene encodes a peroxidase proprotein with a putative 25 amino acid signal sequence. The predicted mature protein is 26.2 kD with a pl of 8.71 and contains the three peroxidase signature sequences at amino acid positions 56 to 73, 114 to 151, and 184 to 194 (Buffard et al., 1990). The predicted mature protein also contains the eight Cys residues involved in disulfide bridges and the two His residues involved with binding heme, both of which are characteristic of plant peroxidases (Morgens et al., 1990). The deduced amino acid sequence of this clone, λ*Shpx6*, described herein (Table I) has 80% homology to the deduced amino acid sequence of the cDNA *Shpx6* and 90 and 48% homology to protein sequences of the peanut peroxidase Pnc1 (Buffard et al., 1990) and wheat peroxidase POX1 (Hertig et al., 1991), respectively. The λ*Shpx6* gene sequence reported is 1848 nucleotides long with two introns at nucleotide positions 212 to 670 and 868 to 1293. This gene may provide a useful tool for the analysis of host pathogen signaling and host gene regulation in *S. humilis* infected by *C. gloeosporioides*.

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Table 1. Characteristics of the *S. humilis* cationic peroxidase gene

| Organism: | *Stylosanthes humilis* cv Paterson (common name: Townsville stylo). |
| Function: | Gene encodes a cationic peroxidase isoenzyme (donor: hydrogen-peroxide oxidoreductase; EC 1.11.1.7). |
| Source: | Genomic library in AEMBL3, constructed from DNA isolated from leaf tissue was constructed using the packaging mix Gigapack II (Stratagene) and the host *Escherichia coli* strain ER1648 (Graham et al., 1990). DNA isolated from *S. humilis* was highly methylated and was degraded by methylation-specific nucleases found in standard *E. coli* K12 host strains. A library with a total of 4.1 × 10^7 plaque-forming units was obtained using *E. coli* ER1648 and the packaging mix Gigapack II (Stratagene). |
| Techniques: | Various restriction fragments were subcloned into pUC18 (New England Biolabs) and sequenced using the Applied Biosystems PRISM DyeDeoxy Terminator Cycle Sequencing Kit and the Applied Biosystems 373A automated sequencing instrument. Synthetic oligonucleotide primers were used to ensure that both strands were sequenced completely. |
| Features of Gene: | The gene sequence is 1848 nucleotides long with two introns at nucleotide positions 212 to 670 and 868 to 1293. The reported sequence includes 300 nucleotides upstream of the putative ATG translational start including putative TATA and CAAT sequences at positions −169 and −216, respectively, from the ATG translational start. |
| Features and Identification of Deduced Protein: | Encodes a 318-amino acid residue proprotein with a putative 25-amino acid signal sequence. The predicted mature protein is 26.2 kD with a pl of 8.71. The predicted protein contains the three peroxidase signature sequences at amino acid positions 56 to 73, 114 to 151, and 184 to 194. The predicted mature protein also contains the eight Cys residues involved in disulfide bridges and the two His residues involved with binding heme in peroxidase enzymes (Morgens et al., 1990). |

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LITERATURE CITED

Curtis MD, Cameron DF, Manners JM (1995) Molecular evidence that the diploid \textit{Stylosanthes humilis} and the diploid \textit{Stylosanthes hamata} are the progenitor of allotetraploid \textit{Stylosanthes hamata} cv. Verano. Genome (in press)