

Molecular Cloning of Peroxidase cDNA from Flower Bud of Ginseng(*Panax ginseng*)

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ABSTRACT

A full-length cDNA (PPrx1) encoding peroxidase has been isolated and its nucleotide sequence determined from flower bud in ginseng plant (*Panax ginseng*). A *PPrx1* cDNA is 1192 nucleotides long and has an open reading frame of 1062 bp with a deduced amino acid sequence of 354 residues (pI 7.53). The deduced amino acid sequence of *PPrx1* matched to the previously reported peroxidase protein genes. The *PPrx1* showed a high similarity with the 64% identity with peroxidase of *N. tabacum* (AAK52084). In the phylogenetic analysis based on the amino acid residues, the *PPrx1* was closer with peroxidase of *G. max* (AAD37376).

Key words : cDNA, flower bud, *Panax ginseng*, peroxidase

INTRODUCTION

Peroxidases perform essential metabolic roles in most living organisms. Their functions include detoxification of hydrogen peroxide, generation of antimicrobial compounds and other contributions to a variety of biosynthetic process. These enzymes are widely distributed among plants, animals and microorganisms (Everse *et al.*, 1991).

Plant peroxidases exist as a large family of isozymes and they have been implicated in a broad range of

physiological processes (Hiraga *et al.*, 2001). The cell wall appears to be a major site for defense related peroxidase polymerization reactions such as lignification (Hammerschmidt and Kuc, 1982), suberization (Espelie *et al.*, 1986), cross-linking of structural cell wall proteins (Bradley *et al.*, 1992), and dimerization of ferulate esters (Ikegawa *et al.*, 1996). These classical peroxidases belong to class III of the plant peroxidase superfamily (Welinder, 1992) and are secretory enzymes found in the extracellular space or in

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the vacuole. They possess a signal peptide necessary for routing to the endoplasmic reticulum, and some of them possess also a C-terminal extension which may be responsible for vacuolar targeting, as has been shown for some plant proteins (Neuhaus, 1996).

During the last decade, several molecular biology approaches have been developed to isolate, characterize and study the expression of peroxidase genes in plants. In *A. thaliana*, since the characterization of the first genes by Intaprak *et al.* (1991), numerous genes encoding peroxidases have been identified through the different expressed sequence tag (EST) sequencing projects and have already been reported (Capelli *et al.*, 1996; Tognolli *et al.*, 2000). In the present study we describe the cDNA cloning, and molecular characterization of *PPrx1* from flower bud of *Panax ginseng*.

MATERIALS AND METHODS

Four-year old *Panax ginseng* plants grown at field were used. Flower buds attached in the upper region were cut with a scalpel and immediately frozen in liquid nitrogen and stored at -80°C further use.

RNA purification and cDNA library construction

Total RNA was isolated from ginseng flower buds using aqueous phenol extraction procedure as described by Morris *et al.* (1990). Tissues were frozen and ground in liquid nitrogen prior to extraction of RNA. Poly (A)⁺ RNA was isolated by oligo (dT) cellulose column using the Poly(A) Quick mRNA isolation kit (Stratagene, US). A commercial cDNA synthesis kit was used to construct library according to the manufacture's instruction manual (Clontech, US). To produce single-stranded cDNA appropriate for directional cloning 5 µg of poly (A)⁺ RNA was primed with an oligo (dT) primer. Double-stranded cDNA was produced using RNase H and *E. coli* DNA polymerase. After ds cDNA

synthesis by primer extension, cDNA was digested with Sfi I and then size-fractionated through a Chroma spin-400 column. Size-selected cDNA was ligated into λ TriplEx2 vector and was packaged *in vitro* using Gigapack III Gold Packaging Extract kits (Stratagene, US). Fractions containing cDNA greater than 500 bp were recovered and this library was amplified once to yield a final titre of 2×10^9 pfu ml⁻¹.

Sequence analysis

The pTriplEx phagemids for peroxidase cDNA were excised from the λTriplEx2 and used as templates for sequence analysis. The cDNA insert was sequenced using the 5' and 3' sequencing primer by an automatic DNA sequencer (ABI prism 3700). Nucleotide and amino acid sequence analyses were performed using DNASIS program (Hitachi). Comparison of sequences to DNA and protein databases at NCBI was performed using the blast algorithm of Altschul *et al.* (1990).

Sequence alignment and phylogenetic analysis

We used Clustal W (1.82) with default gap penalties to perform multiple alignment of peroxidases isolated in ginseng and previously registered in other plants (Thompson *et al.*, 1994). Based on this alignment, a phylogenetic tree was constructed according to the UPGMA method.

RESULTS AND DISCUSSION

Korean ginseng (*Panax ginseng* C. A. Meyer) is one of the most medicinally important herb. Ginseng has many beneficial bioactive effects on human health. However, cultivation and breeding of the plant is very difficult, because it requires at least 4-year cultivation period for root harvest. Ginseng plant during the long cultivation time endure against various environmental stresses and pathogen attack. To acquire the gene expression profiling in the flower bud of ginseng, we

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GGAACACCAAAGAGAATCAACACAATGGCTTCTACTTTAACTTCTTTGAGCTCTCTCTT   60
      M A S T L T S L S S L F
CITCITTTTTCTCTTCTCTTTCACTTCTACTTTACCACTTGCATGTCTCAGAGGCACAAAC   120
      F F F S S L S L L L Y H L H V S E A Q T
TCCACCTATAGTGAAGGGACTTTTCATTGGCTTTCTTTGATTCTACCTGTCTTAAGCTTGA   180
      P P I V K G L S L A F F D S T C P K L E
ATCCATTGTTAGGAAACAGCTTGAGAAAGAGITCAAGGCTGATATCGGCCAAGCTGCTGG   240
      S I V R K Q L E K E F K A D I G Q A A G
ATTGCTCCGACTCCACTTCCATGATTGCTTTGTCCAGGGATGTGATGCATCGGTTTTGCT   300
      L L R L H F H D C F V Q G C D A S V L L
CGACGGTTCCGCCAGTGGACCATCGGAAAAGAATGCACCTCCAACCTGAGTTTGAGGGC   360
      D G S A S G P S E K N A P P N L S L R A
AAAGGCATTACGATCATCGAAGATCTCCGTCGACAAGTGCACAAGCAATGCGGTAATAAT   420
      K A F T I I E D L R R Q V H K Q C G K I
TGTCTCCTGTGCCGATATCACTGCTCTTGCTGCCCGAGATGCAGTTGTTTTGTCCGGTGG   480
      V S C A D I T A L A A R D A V V L S G G
CCCAAACACCAAGTCCCATACGGAAGACGCGACGGTCTCCAATTTGCAACCAGACAGGC   540
      P N Y Q V P Y G R R D G L Q F A T R Q A
CACACTAGCCAACCTTCTCCACCATTTGCCAATACAACCACCATCCTCAACTCCCTTGT   600
      T L A N L P P P F A N T T T I L N S L V
CACAAAAAATTTTCGACCCTACCGACGTGGTTCGCGCTATCTGGGGCCACACAATTGGACT   660
      T K N F D P T D V V A L S G A H T I G L
TAGCCATTGTAGTTCTTTTATTCGTGCTTATTCGACCCCAAGACTCTACAATGGCGCA   720
      S H C S S F I R R L F P T Q D S T M A Q
AAGCTTTGCAAAAGACCTCAGAATCACTTGCCAACAATAACAACCGACAACACAATAA   780
      S F A K D L R I T C P T N T T D N T T N
CCTAGACTTTGCAACTCCAATGTGTTGATAACAAGTATTATGTGGACCTTGTTAATCG   840
      L D F R T P N V F D N K Y Y V D L V N R
ACAAGGGCTGTTTACGTCTGACCAGGACTTGTTCACTGATAATAGGACTCGGGGCATTGT   900
      Q G L F T S D Q D L F T D N R T R G I V
TACTAGTTTTGCTAATAATCAGACCTTGTTTTTCGAGAAGTTTGTGAATGCAATGTTAAA   960
      T S F A N N Q T L F F E K F V N A M L K
AATGGGGCAGTTGAGCGTGTGACGGGCACACAAGGGGAGATTGAGGAAATGTTTCGGT   1020
      M G Q L S V L T G T Q G E I R G N C S V
TAAGAATTCGAATAATTTGTTCTTGTCAACTGTGGTGGAAAGAGGGGATGGAGAATTTGTT   1080
      K N S N N L F L S T V V E E G M E N L L
AGGATTTTGAGGGTGACTATGGGTGTTATTATGTAATCTTGCTGATTGTGCGTTACCTT   1140
      G F *
TTGTATGTTTTTGTGTTTCAAGCCTTTGAAAATAAAGTAGTCTTTTTGACC           1192

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Fig. 1. Nucleotide and deduced amino acid sequence with the open reading frame from 26 to 1090. The positions of nucleotides are shown on the left and the positions of amino acids under the below. Asterisk shows the termination codon. The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequencing Database under the accession number AB232686.

Panax	MASLTSLSSLFFFSSLSLLLYHLHVSEAQT-PP VKGLSLAFFDSTCPKLES VRKQL	59
Glycine	-----ARSSAYPLFL SSILF SHIYASQVEAKPPVVKGLSYSFYSKTCPKLES VRKHL	55
Nicotiana	-----MASITLSSFAASLLL LSVNFYQTEAAGTQP VKGLSWTFYDS CPNAES IRSR	56
Spinacia	-----MAATKG-----FPL LVLSL IGLSQGGST PVVPGLSYTFYSSSCPGLDF IRGHL	53
Linum	-----MAPTSSHVVALT IMLSAVLFASTTTAQ PAPAKGMSWTFYKSSCPKLES ITKRL	55
Euphorbia	-----MASKLVLVSCLLVAFWFC EAQTKPP VNLGSWTFYKSSCPKVES IQKEL	52
Triticum	-----MARAPLLAALVVAVVVASSLGHGASAAEPPVARGLSFDFYRRTCPRAES VRGFV	55
	: : : : : * : * : * : * : :	
Panax	EKEFKAD GQAAGLLRLHFHDFCVQGCDAVLLDGSASGP-SEKNAPPNLSLRKAFT	118
Glycine	KKVFKDDNGQAPALLR FFHDFCVQGCDSLLLDGS-----P-SERDQPANGG RTEALQT	111
Nicotiana	QQVFRQD GQAAGLLRLHFHDFCVQGCDSVLLDGSASGP-SEKDAPPNLTLRQAQFR	115
Spinacia	WQ FQSDLTQAAGLLRLHFHDFCVQGCDSVLLDGSASGP-SEKEAPPNLTLRQAQFR	112
Linum	KEVFKKD GQAAGLLRLHFHDFCVQGCDSVLLTGSAGGPSAEQGSPPNLSLRKAFT	115
Euphorbia	KKLFFKQVEQAAGLLRLHFHDFCVQGCDSVLLNGSAGGP-SEQSELNLSLRQAQFR V	111
Triticum	QDAVRKD GLAAGLLRLHFHDFCVQGCDAVLLDGSATGP-GEQQAPPNLTLRPSAFKAV	114
	: : * * : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
Panax	EDLRRQVHKQC-GK VSCAD TALAARDAVVLGGPNYQVPYGRDGLQFATRQATLANL	177
Glycine	DD RA IHKEC-GRI VSCAD TVLAARDSVFLTGGPDYAVPLGRDGLSFST-SGTSDL	168
Nicotiana	EDLRRRVHRDC-GRVVSAD TA AARDSVFLGGPDYDLPLGRDGLNFATRNETLANL	174
Spinacia	NDLRALVHQQC-GRVVSAD TALAARESVFLAGGPFYVWPLGRDGLNFATLSDTLANL	171
Linum	DDLRAVHKQC-GRVVSAD VALAARDSVVLGGPKYQVALGRDGTTLVTQDTTLANL	174
Euphorbia	NDLRALVHKEC-GPVVSCSD VA AARDSVFLTGGPKYDVPPLGRDGVKFAEVNATFEHL	170
Triticum	ND RDRLERECRGAUVSCAD LALAARDSVVVSGGPDYRVPPLGRDSSRFATRQDVLSDL	174
	: * : * : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
Panax	PPPFANTTT LNSLVTKNFDPDVALSGAHT GLSHCSSF RRLFPTQDSTMAQSFADK	237
Glycine	PKPFNTTGVTLDAFAAKNFDPDVALSGAHTFGRAHCGTFFNRLSP-LDPNMDKTLAKQ	227
Nicotiana	PPPSFNASAL TSLATKNFTPTDVALSGGHT G GHCTSFTERLYPNQDPSMDKTFANN	234
Spinacia	PPPSFNTQQLDLSLANKLNATDLVALSGGHT G SHCTSFTDRLYPTQDPTMAQTFANN	231
Linum	PPPFATTGT LSSLATKNLNPTDAVALSGAHT G SHCSSFTDRLYPNQDPSMDQTFAKN	234
Euphorbia	VGPTANVTT LAKLARKGLD TTDAVSLSGGHT G GHCTSFTERLYPSQDPTLTKTFANN	230
Triticum	PAPSSNVPSLLALLRPLGLD ATLVT ISGGHT GQAHCSSFEDRLFRPDPPT NPPFLAR	234
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Panax	LR TCPTNTTNTNLD FRTPNVFQNKYYVQVLDVNRQGLFTSDQDLFTDNRTRG VTSFAN	297
Glycine	LQSTCPDANSNGNTANLD RTPTVFNKYVLDL MNRRQGVFTSDQDLNDKRTKGLVNAFAL	287
Nicotiana	LKNTCPTSNSNTTTLVD IRSPNKFQNKYYVQVLDL MNRRQGLFTSDQDLYDTRRTRG VTSFAI	294
Spinacia	LKVTCPATTNATTNLD IRTPNVFQNKYY DLMNRQGLFTSDQDLYDTSRTKD VTSFAL	291
Linum	LKATCPQAATTDN- VD IRSPNVFQNKYYVQVLDL MNRRQGLFTSDQDLYDTSRTG VTSFAI	293
Euphorbia	LKRTCPNVNTENSTFLD LATPNEFDNRYVQVLDL MNRRQGLFTSDQDLYDTRTRQ V DFAV	290
Triticum	LKGTCPAKGTDRTVLD VRTPNVFNQNKYYVQVLDVNR EGLFVSDQDLFTND TRP VERFAR	294
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
Panax	NQTLFFEKFNAMLKMGQLSVLTGTQGE RGNCVSKNS-----NN-----LFLSTVVEEGM	348
Glycine	NQTLFFEKFDAT KLSQLDVL TGNQGE RGKCNVNA-----RK-----SLLTSVVEEVV	338
Nicotiana	NESLFFEEFVNSM KMGQLNVL TGTQGE RANCSVRNS-----ANYNLLSTSVAAEQ-Q	348
Spinacia	NQNLFFQKF DAMVKGQLNVL TGTQGE RANCSVRN-----ANSNLHLKSVVE DQEQ	345
Linum	NQTLFFEKVVAM KMGQ SVLTGTQGE RANCSVTNS-----AKVQTSFLEEAVEEV	348
Euphorbia	NQTLFYEFK IGM KMGQLEVTGNQGE RNDCSFRNS-----D-----NYLVSVDDEES	340
Triticum	SQRDFFEQFGVSMGKMGQMRVRTSDLGEVRRNCSARNPGPAAADELQWPSFVQT VDAAA	354
	: : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
Panax	ENLLGF-	354
Glycine	QLVDQF-	344
Nicotiana	RTWSEI-	354
Spinacia	QTLSEQ-	351
Linum	ELLAGMR	355
Euphorbia	GSSSELR	347
Triticum	ERLG-	358

Fig. 2. Alignment of the amino acids residues of *PPrx1* (AB232686) with those of peroxidase genes from other plants; *N. tabacum* (AAK52084), *L. usitatissimum* (AAB02926), *S. oleracea* (CAA71490), *E. characias* (AAS97959), *G. max* (AAD37376), and *T. aestivum* (AAM88383).

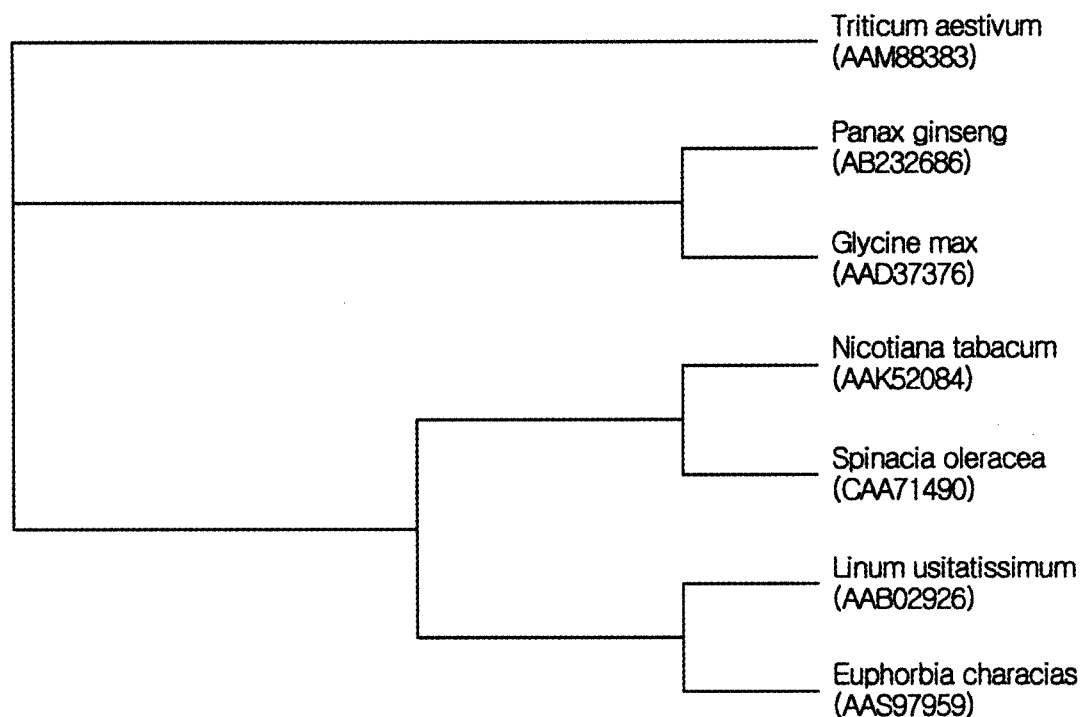


Fig. 3. Phylogenetic relationship of the peroxidase protein family from *P. ginseng* and other plants. Phylogenetic analysis is based on the deduced amino acid sequences of peroxidase genes from various plant species. The branch lengths are proportional to divergence, with the scale of 0.1 representing 10% change.

performed expressed sequence tags (EST) analysis.

A full-length cDNA library of flower bud was constructed using 5 μ g of poly (A)+mRNA. Generated ginseng flower bud ESTs were compared against all sequences in the nonredundant database at the NCBI using the program BLASTX, which compares translated nucleotide sequences with protein sequences. A full-length peroxidase protein homolog of analyzed EST clones was isolated and full sequenced and named with *PPrx1*. The *PPrx1* gene was 1192 nucleotides long and possess an open reading frame of 1062 bp with 25 bp 5'-untranslated region (5'UTR) and 102 bp 3' UTR (Fig. 1). BLASTX database searches with the *PPrx1* sequence gave peroxidase proteins from various organism as the top 100 best matches. The *PPrx1* cDNA encodes a protein of 354 amino acids with a predicted molecular mass 38814 Da (pI 7.53) (Fig. 1).

The deduced amino acid sequence of *PPrx1* cDNA was compared to other related peroxidase gene products registered previously (Fig. 2). The *PPrx1* protein sequence shared the highest similarity (64%) with *N. tabacum* (AAK52084), followed by 63 % with *L. usitatissimum* (AAB02926). We compared the phylogenetic relationships of the *PPrx1* with those of the eukaryotic peroxidase protein registered previously (Fig. 3). The *PPrx1* was clustered with ribosomal protein of *G. max* (AAD37376).

We have isolated and characterized of peroxidase gene from flower bud in ginseng plant. Peroxidase is very important protein for defense against pathogen attack, salt tolerance and oxidative stress. Further characterisation of ginseng peroxidase gene is required to understand their physiological roles in the flower bud of *P. ginseng*.

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