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

Jun-Gyo In, Bum-Soo Lee, Won-Seob Song[1], Chang-Hyu Bae[1], Seong-Kyu Choi[2], Deok-Chun Yang[3]*

Biopia Co., Ltd, Yongin 449-598, Korea

[1] College of Agriculture and Life Sciences, Sunchon National University, Sunchon 540-742, Korea

[2]Department of Oriental Medicine Resources, College of Nature Science, Sunchon National University, Suncheon 540-742, Korea

[3]Department of Oriental Medicinal Materials and Processing, Kyung Hee University, Suwon 449-701, Korea

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

*Corresponding author : **Deok Chun Yang**, E-mail: dcyang@khu.ac.kr

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 Intapruk *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℃ 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 $m\ell^{-1}$.

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 penealties 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

```
GGAACACCAAAAGAGAATCAACACAATGGCTTCTACTTTAACTTCTTTGAGCTCTCTCTT      60
                          M  A  S  T  L  T  S  L  S  S  L  F

CTTCTTTTTCTCTTCTCTTTCACTTCTACTTTACCACTTGCATGTCTCAGAGGCACAAAC     120
 F  F  F  S  S  L  S  L  L  L  Y  H  L  H  V  S  E  A  Q  T

TCCACCTATAGTGAAGGGACTTTCATTGGCTTTCTTTGATTCTACCTGTCCTAAGCTTGA     180
 P  P  I  V  K  G  L  S  L  A  F  F  D  S  T  C  P  K  L  E

ATCCATTGTTAGGAAACAGCTTGAGAAAGAGTTCAAGGCTGATATCGGCCAAGCTGCTGG     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

CGACGGTTCCGCCAGTGGACCATCGGAAAAGAATGCACCTCCAAACCTGAGTTTGAGGGC     360
 D  G  S  A  S  G  P  S  E  K  N  A  P  P  N  L  S  L  R  A

AAAGGCATTCACGATCATCGAAGATCTCCGTCGACAAGTGCACAAGCAATGCGGTAAAAT     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

CCCAAACTACCAAGTCCCATACGGAAGACGCGACGGTCTCCAATTTGCAACCAGACAGGC     540
 P  N  Y  Q  V  P  Y  G  R  R  D  G  L  Q  F  A  T  R  Q  A

CACACTAGCCAACCTTCCTCCACCATTTGCCAATACAACCACCATCCTCAACTCCCTTGT     600
 T  L  A  N  L  P  P  P  F  A  N  T  T  T  I  L  N  S  L  V

CACAAAAAATTTCGACCCTACCGACGTGGTCGCGCTATCTGGGGCCCACACAATTGGACT     660
 T  K  N  F  D  P  T  D  V  V  A  L  S  G  A  H  T  I  G  L

TAGCCATTGTAGTTCTTTTATTCGTCGCTTATTCCCGACCCAAGACTCTACAATGGCGCA     720
 S  H  C  S  S  F  I  R  R  L  F  P  T  Q  D  S  T  M  A  Q

AAGCTTTGCAAAAGACCTCAGAATCACTTGTCCAACAAATACAACCGACAACACAACTAA     780
 S  F  A  K  D  L  R  I  T  C  P  T  N  T  T  D  N  T  T  N

CCTAGACTTTCGAACTCCAAATGTGTTCGATAACAAGTATTATGTGGACCTTGTTAATCG     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

AATGGGGCAGTTGAGCGTGTTGACGGGCACACAAGGGGAGATTCGAGGAAATTGTTCGGT    1020
 M  G  Q  L  S  V  L  T  G  T  Q  G  E  I  R  G  N  C  S  V

TAAGAATTCGAATAATTTGTTCTTGTCAACTGTGGTGGAAGAGGGGATGGAGAATTTGTT    1080
 K  N  S  N  N  L  F  L  S  T  V  V  E  E  G  M  E  N  L  L

AGGATTTTGAGGGTGACTATGGGTGTTATTATGTAATCTTGCTGATTGTGCGTTACCCTT    1140
 G  F  *

TTGTATGTTTTTGTTGTTTCAAGCCTTTGAAAATAAAGTAGTCTTTTTGACC            1192
```

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.

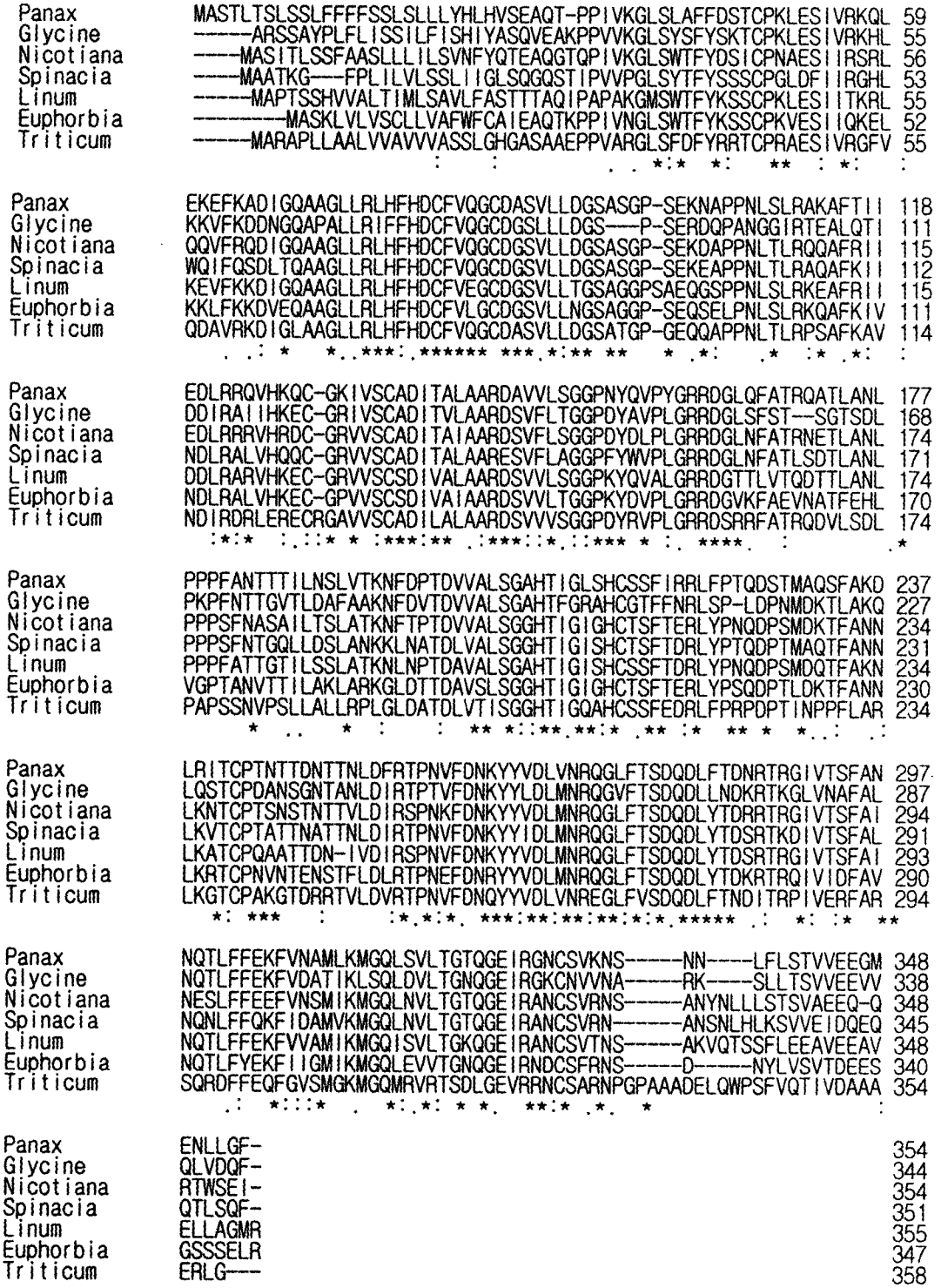

Fig. 2. Alignment of the amino acids residues of *PPrx1* (AB232686) with those of peroxidase genes from other plants; N. tabacum (AAK52084), L. usitatissimum (AAB02926), S. oleracea (CAA71490), E. characias (AAS97959), G. max (AAD37376), and T. aestivum (AAM88383).

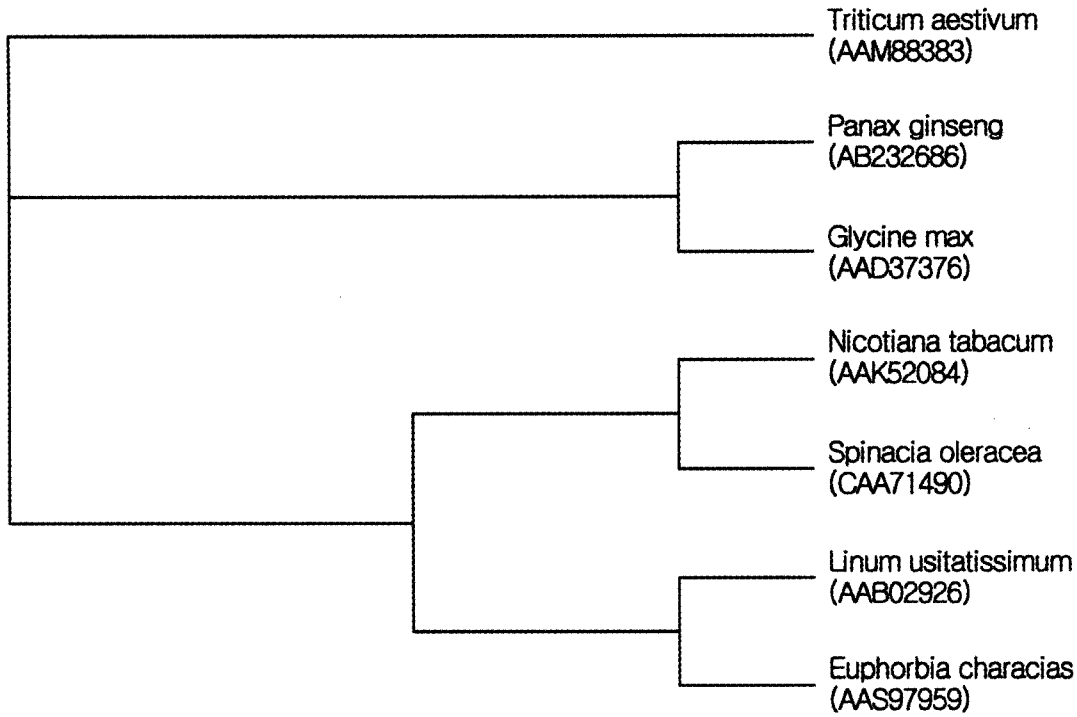

Fig. 3. Phylogenetic relationship of the peroxidase protein family from P. ginseng and other plants. Phylogenetic analysis is based on the deduced amino acid sequences of peroxidase genes from various plant species. The branch lengths are proportional to divergence, with the scale of 0.1 representing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

performed expressed sequence tags (EST) analysis.

A full-length cDNA library of flower bud was constructed using 5μg 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*.

ACKNOWLEDGEMENTS

This work was supported by a grant of ARPC in Korea(Project # : 203055-03-2-CG000).

LITERATURE CITED

Altschul, S.F., W. Gish , W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Bradley, D.J., P. Kjellborn and C.J. Lamb. 1992. Elicitor-and wound induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell 70:21-30.

Capelli, N., M. Tognolli, J. Flach, S. Overney, C. Penel, H. Greppin and P. Simon. 1996. Eleven cDNA clones from *Arabidopsis thaliana* encoding isoperoxidases (Accession Nos X98313, X98314, X98315, X98316, X98317, X98318, X98319, X98320, X98321, X98322, and X98323). Plant Physiol. 112:406.

Espelie, K.E., V.R. Franceschi and P.E. Kolattukudy. 1986. Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound-healing potato tuber tissue. Plant Physiol. 81:487-492.

Everse, J., K.E. Everse and M.B. Grisham. 1991. Peroxidases in Chemistry and Biology. CRC press, Boca Raton, FL.

Hammerschmidt, R. and J. Kuc. 1982. Lignification as a mechanism for induced systemic resistance in cucumber. Physiol. Plant Pathol. 20: 61-71.

Hiraga, S., K. Sasaki, H. Ito, Y. Ohashi and H. Matsui. 2001. A large family of class III plant peroxidases. Plant Cell Physiol. 42:462-468.

Ikegawa, T., S. Mayama, H. Nakayashiki and H. Kato. 1996. Accumulation of diferulic acid during the hypersensitive response of oat leaves to *Puccinia coronata* f. sp. avenae and its role in the resistance of oat tissues to cell wall degrading enzymes. Physiol. Mol. Plant Pathol. 48: 245-255.

Intapruk, C., N. Higashimura, K. Yamamoto, N. Okada, A. Shinmyo and M. Takano. 1991. Nucleotide sequences of two genomic DNAs encoding peroxidase of Arabidopsis thaliana. Gene 98:237-241.

Morris, P.C., A. Kumar, D.J. Bowles and A.C. Cuming. 1990. Osmotic stress and abscisic acid regulate the expression of the Em gene of wheat. Eur. J. Biochem. 190:625-630.

Neuhaus, J.M. 1996. Protein targeting to the plant vacuole. Plant Physiol. Biochem. 34:217-221.

Thompson, J.D., D.G. Higgins and T.J. Gilbson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-secific gap penalties and weight matrixchoice. Nucleic Acids Res. 22:4673-4680.

Tognolli, M., S. Overney, C. Penel, H. Greppin and P. Simon. 2000. A genetic and enzymatic survey of Arabidopsis thaliana peroxidase. Plant Perox. Newslett. 14:3-12.

Welinder, K.G. 1992. Superfamily of plant, fungal and bacterial peroxidases. Curr. Opin. Struct. Biol. 2, 388-393.

(Received May. 21, 2005)
(Accepted Aug. 4, 2005)