cDNA cloning and expression analysis of a mannose-binding lectin from *Pinellia pedatisecta*

Juan Lin[a], Xuanwei Zhou[b], Shi Gao[a], Xiaojun Liu[a], Weisheng Wu[a], Xiaofen Sun[a] and Kexuan Tang [a,b,*]

[a]*State Key Laboratory of Genetic Engineering, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, School of Life Sciences, Morgan-Tan International Center for Life Sciences, Fudan University, Shanghai 200433, People's Republic of China*

[b]*Plant Biotechnology Research Center, School of Agriculture and Biology, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, School of Life Sciences and Technology, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China*

[*]*Corresponding author (Fax, +86-21-65643552; Email, kxtangl@yahoo.com)*

Pinellia pedatisecta agglutinin (PPA) is a very basic protein that accumulates in the tuber of *P. pedatisecta*. PPA is a hetero-tetramer protein of 40 kDa, composed of two polypeptide chains A (about 12 kDa) and two polypeptides chains B (about 12 kDa). The full-length cDNA of PPA was cloned from *P. pedatisecta* using SMART RACE-PCR technology; it was 1146 bp and contained a 771 bp open reading frame (ORF) encoding a lectin precursor of 256 amino acid residues with a 24 amino acid signal peptide. The PPA precursor contained 3 mannose-binding sites (QXDXNXVXY) and two conserved domains of 43% identity, PPA-DOM$_1$ (polypeptides A) and PPA-DOM$_2$ (polypeptides B). PPA shared varying identities, ranging from 40% to 85%, with mannose-binding lectins from other species of plant families such as Araceae, Alliaceae, Iridaceae, Liliaceae, Amaryllidaceae and Bromeliaceae. Southern blot analysis indicated that *ppa* belonged to a multi-copy gene family. Expression pattern analysis revealed that *ppa* expressed in most tested tissues, with high expression being found in spadix, spathe and tuber. Cloning of the *ppa* gene not only provides a basis for further investigation of its structure, expression and regulatory mechanism, but also enables us to test its potential role in controlling pests and fungal diseases by transferring the gene into plants in the future.

[Lin J, Zhou X, Gao S, Liu X, Wu W, Sun X and Tang K 2007 cDNA cloning and expression analysis of a mannose-binding lectin from *Pinellia pedatisecta*; *J. Biosci*. **32** 241–249]

1. Introduction

Many plants contain carbohydrate-binding proteins that are commonly known as lectins, agglutinins or haemagglutinins. Mannose-binding lectins are a group of newly discovered plant lectins, and are newly discovered. By virtue of their unique carbohydrate-binding properties, mannose-binding lectins have become very useful tools in biomedical and glycoconjugate research, and are also extensively used to study the infection cycle of the human immunodeficiency virus (HIV) and chemotherapy of acquired immunodeficiency syndrome (AIDS; Van Damme *et al* 1995). Mannose-binding lectins have been characterized and cloned from eight families of angiosperms, such as Alliaceae, Amaryllidaceae, Orchidaceae, Bromeliaceae, Liliaceae, Araceae, Iridaceae and Zingiberaceae (Van Damme *et al* 2000; An *et al* 2006; Wright *et al* 1999; Smeets *et al* 1997a; Neuteboom *et al* 2002; Balzarini *et al* 2004; Chen *et al* 2005) and one family

Keywords. *Pinellia pedatisecta*; *Pinellia pedatisecta* agglutinin (PPA); cDNA cloning; mannose-binding lectin; RACE; expression pattern

Abbreviations used: ORF,open reading frame; RACE, rapid amplification of cDNA ends; PPA, *Pinellia pedatisecta* agglutinin; PCR, polymerase chain reaction; pI, isoelectric point; RT-PCR,reverse transcriptase polymerase chain reaction.

of gymnosperm, Taxaceae (Kai *et al* 2004). The majority of cloned lectin genes are one-domain lectins that synthesize as protomer; these are converted into mature lectin polypeptides by the co-translational cleavage of a signal peptide and the post-translational remova1 of a C-terminal peptide (Van Damme *et al* 1991). Apart from the one-domain lectins, there are three types of two-domain protomer forms. The first type is the heterodimer, composed of two different polypeptides, both of which are derived from a single precursor consisting of two very similar tandemly arrayed lectin domains (e.g. *Allium ursinum* agglutinin, AUA; Van Damme *et al* 1993). The second type is the heterotetramer, consisting of two different polypeptides of either identical or slightly different size. The two polypeptides of these lectins are derived from two-domain precursors consisting of two tandemly arrayed lectin domains. In some cases the two domains are highly homologous (e.g. *Allium sativum* agglutinin, ASA; Van Damme *et al* 1992) and in others the homology is much less (e.g. *Arum maculatum* agglutinin, AMA; Van Damme *et al* 1995). The third type is the hetero-octamer, which is a tetramer of four identical subunits of 28 kDa containing two separate domains (e.g. *Tulipa hybrid* tulip lectin, TxLCI; Van Damme *et al* 1996). Mannose-binding lectins are the most reliable for genetic modification of crops with respect to food safety (Van Damme *et al* 1998).

There are abundant mannose-binding lectins in the tube of Araceae species (Van Damme *et al* 1995). *Amorphophallus konjac* agglutinin (AKA; Fei *et al* 2003) and *Zantedeschia aethiopica* agglutinin (ZAA; Chen *et al* 2004) are the only one-domain lectins; all mannose-binding lectins cloned from family Araceae belong to two-domain lectins, such as *Colocasia esculenta* agglutinin (CEA; Hirai *et al* 1993; Bezerra *et al* 1995), *Amorphophallus maculatum* agglutinin (AMA), *Xanthosoma sagittifolium* agglutinin (XSA), *Dieffenbachia sequina* agglutinin (DSA; Van Damme *et al* 1995), *Arisaema heterophyllum* agglutinin (AHA; Lin *et al* 2005a), *Pinellia ternata* agglutinin (PTA; Yao *et al* 2003) and *Arisaema lobatum* agglutinin (ALA; Lin *et al* 2005b).

Pinellia pedatisecta agglutinin (PPA) is a very basic protein accumulated in the tuber of *P. pedatisecta*, and is used in Chinese traditional medicine. Native PPA is a heterotetramer protein of 40 kDa, composed of two polypeptides A (about 12 kDa) and two polypeptides B (about 12 kDa). Polypeptides A and polypeptides B are slightly different in size and very different in pI (4.3–8.15) (Sun *et al* 1995; Huang *et al* 1997). Native PPA is a glycoprotein containing mannose, fucose and N-acetylglucosamide. Earlier studies showed that native PPA exhibited various pharmacological and biological activities including termination of pregnancy (Tao *et al* 1981) and anti-tumour activity (Sun *et al* 1992; Zhu *et al* 1999).

Recent insect bioassay studies showed that native PPA had significant insecticidal activity against cotton aphids (*Aphis gossypii* Glover) and peach potato aphids (*Myzus persicae* Sulzer), and the insecticidal activity of native PPA was very similar to that of *Galanthus nivalis* agglutinin (GNA; Huang *et al* 1997; Pan *et al* 1998), making PPA a potential candidate for controlling aphids by genetic engineering. In this paper, we report the cloning and characterization of the full-length cDNA of the lectin gene from *P. pedatisecta*, which provides useful information not only on the structure of the gene, but also on the regulation of its expression.

2. Materials and methods

2.1 *Plant materials*

Tubers of *P. pedatisecta* were collected from Jinhua, Zhejiang province, China. The tubers were grown in pots in the greenhouse under standard conditions. Root, leaf, spadix, petiole, spathe, bud and tuber were collected and stored at –70°C until use.

2.2 *RNA extraction*

Total RNA was extracted from *P. pedatisecta* tissues by the cetyltrimethyl ammonium bromide (CTAB)-based RNA isolation method (Jaakola *et al* 2001). RNA quality and concentration were checked by agarose gel electrophoresis (EC250-90, E-C Apparatus Corporation) and spectrophotometer analysis (WFZUV-2100, Unico™ Instruments Inc.), and the RNA samples were stored at –70°C until use.

2.3 *3′ and 5′-RACE of ppa*

cDNA synthesis was performed with SMART technology (SMART™ RACE cDNA Amplification Kit) for the rapid amplification of cDNA 5′- and 3′- end (RACE; CLONTECH Laboratories, Inc., Palo Alto, California, USA). For 3′-RACE, RNA was reversely transcribed with the 3′-RACE CDS Primer A (provided in the kit), and was performed with primer 1 (table 1), and designed and synthesized (Shanghai Sangon Biotechnological Company) based on the mannose-binding site sequence (MQDDCNL) conserved among most of the monocot mannose-binding lectins. For 5′-RACE, RNA was reversely transcribed with the 5′-RACE CDS Primer and SMART II A Oligonucleotide (provided in the kit). Based on the sequence of the 3′-RACE product, the specific primers 2 and 3 were designed and synthesized. The first round of PCR was performed with primer 2 and Universal Primer A Mix (UMP; provided in the kit). The PCR product was diluted 50-fold for the second round of

Table 1. Primers used in the cloning of *ppa* by rapid amplification of cDNA ends (RACE).

Primer	Primer sequence (5′→ 3′)
3′-RACE CDS primer A	5′-AAGCAGTGGTATCAACGCAGAG TAC(T)30N_{-1}N-3′ (N=A,C,G, or T; N_{-1} = A, G, or C)
5′-RACE CDS primer	5′-$(T)_{25}N_{-1}$N-3′
SMARTII A Oligo	5′-AAGCAGTGGTATCAACGCAG AGTA-3′
UPM	5′-CATATACGACTCACTATAG GGC-3′ (Short, 0.4μM) and 5′-CTAATAC GACTACTATAGGGCAAGCAGTGGTA TCAACGCAGAGT-3′ (Long, 2μM)
NUP	5′-AAGCAGTGGTATCAACGCAG AGT-3′
Primer 1	5′-ATGCAGGATGACTGCAACCT-3′
Primer 2	5′-TCATGCACGATTATGTCCGTG-3′
Primer 3	5′-TTGGAGCTGGATCGGCTGCTC-3′
Primer 4	5′-CAGCCAGTAACACGGCGAAC AAG-3′
Primer 5	5′-GATCAAGGTAGCCATACATATTA-3′

amplification with primer 3 and Nested Universal Primer A (NUP; provided in the kit). The 5′- and 3′-RACE were performed essentially according to the SMART™ RACE cDNA Amplification Kit user manual. All the primers used in RACE are listed in table 1.

2.4 *Full-length cDNA amplification of ppa*

By aligning the 3′-RACE and 5′-RACE product sequences on Vector NTI™ Suite 8.0, the full-length cDNA of *ppa* was deduced and amplified using gene-specific primers 4 and 5 (table 1). Full-length cDNA amplification was performed according to the One Step RNA PCR Kit (AMV) user manual (TaKaRa, China).

All the PCR products were purified using the Gel Extraction Mini Kit (Watson, China), ligated to pMD18-T vectors (TaKaRa, China), transformed into *Escherichia coli* strain DH5α and sequenced with DYEnamic Direct dGTP Sequencing Kit (Amersham Pharmacia, England) and a 373A DNA sequencer.

2.5 *Computer analyses*

The analysis of the deduced amino acid sequence and its comparison with published sequences of mannose-binding lectins were performed with blastp (Standard Protein–Protein BLAST) on the NCBI site (*www. ncbi.nlm.nih.gov*) and the plant lectin database on the lectinDB site (*http: //nscdb.bic.physics.iisc.ernet.in*). The conserved domains were searched with RPS-BLAST (Search the Conserved Domain Database) on NCBI and with PROSITE-Protein families and domains on the ExPASy Proteomics Server. The dendrogram tree was aligned with CLUSTAL W (1.82) using default parameters.

2.6 *Southern blot analysis*

Genomic DNA was extracted from leaf materials of *P. pedatisecta* by the CTAB method (Lin *et al* 2003). Aliquots of genomic DNA (20 μg/sample) were digested overnight at 37°C with *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII, respectively, which did not cut within the probe region. These were then fractionated by 0.8% agarose gel electrophoresis and transferred onto a positively charged Hybond-N^+ nylon membrane (Amersham Pharmacia, England). The open reading frame (ORF) sequence of *ppa* was generated by PCR using primers S1 (5′-AT GGCCTCCAAGCTCCTCCTCTTCC-3′) and S2 (5′-CTCCGCAGCAATGGAGCGCTTCG AG-3′) and used as the probe in Southern blot analysis. Probe labelling (biotin), hybridization and signal detection were performed using the Gene images random prime labelling module and CDP-Star detection module following the manufacturer's instructions (Amersham Pharmacia, England). The membrane was washed under stringent conditions (60°C) and the hybridized signals were visualized by exposure to Fuji X-ray film at room temperature for 2 h.

2.7 *Expression analysis of ppa in* P. pedatisecta *tissues*

Semi-quantitative one-step RT-PCR was used to investigate *ppa* expression profiling in various tissues of *P. pedatisecta*. RNA was extracted separately from seven kinds of tissues including root, leaf, spadix, petiole, spathe, bud and tuber, and used in RT-PCR analysis using a one-step RT-PCR Kit (TaKaRa, China). Amplification for *ppa* was performed at 50°C for 30 min followed by 94°C for 2 min and by 20 cycles of amplification (94°C for 30 s, 58°C for 30 s, 72°C for 50 s) using primers S1 and S3 (5′-CGTTCGAGTGGCGCTC CTTGGTGTA-3′), which amplified a fragment of 500 bp from 92 bp to 591 bp of *ppa*. At the same time, 18S ribosome RNA was also used in RT-PCR in parallel with the *ppa*, using primers 18SF (5′-ATGATAACTCGAC G GATCGC-3′) and 18SR (5′-CTTGGATGTGGTAGCCGTTT-3′), which amplified a fragment of 126 bp from 18S rRNA as the control under the same conditions. The amplified products were analysed with the Gene analysis software package (Gene Company, USA).

3. Results and discussion

3.1 *Cloning and characterization of the full-length cDNA of ppa*

Based on the conserved region of plant mannose-binding lectins, a specific primer (primer 1) was designed and synthesized for the amplification of the 3′-end cDNA of *ppa*. A fragment of about 0.6 kb was amplified in which a 3′ untranslated region of 281 bp was found downstream from the terminal codon. Subsequently, two specific primers designed according to the obtained 3′ RACE fragment sequence (primers 2 and 3) were used for the amplification of the 5′-end cDNA of *ppa*, resulting in a fragment of about 0.75 kb in which a 5′ untranslated region of 91 bp was identified upstream of the first ATG codon. Finally, the full-length cDNA sequence of *ppa* was deduced and amplified through RT-PCR using the gene-specific primers 4 and 5, and confirmed by sequencing. The full-length cDNA of *ppa* was 1146 bp and contained a 771 bp ORF encoding a precursor of 256 amino acids with a calculated molecular weight of 28 kDa and pI of 7.85 (figure 1).

Like most of the mannose-binding lectins from Araceae species, the deduced amino acid sequence of the *ppa* gene contained three mannose-binding sites (QXDXNXVXY). The amino acid sequences of sites I and II were the same as those of GNA. However, the amino acid sequence of site III was different from that of GNA, in which Asp (D) and Asn (N) were substituted by Asn (N) and Phe (F), respectively. A 24-amino acid signal peptide was found in PPA based on the rules of predicting signal peptides (von Heijne *et al* 1986); this was in agreement with reports of most mannose-binding lectins from the Araceae family (Van Damme *et al* 1998; Fei *et al* 2003; figure 1). The signal peptide contained highly hydrophobic amino acid residues (79.2%), implying that it was a secretory putative signal peptide.

3.2 *Homology analysis*

The database search using a plant lectin database (LectinDB) and analysis with blastp showed that the deduced amino acid sequence of PPA had high homology with many existing monocot mannose-binding lectins. Homology was higher with two-domain lectins than with one-domain ones. PPA had higher identity with those of other Arareae species (from 87% to 75%) than with those of Alliaceae, Amaryllidaceae, Orchidaceae, Bromeliaceae, Liliaceae, Araceae, Iridaceae and Zingiberaceae (from 41% to 32%) (figure 2).

According to the NCBI conserved domain search and the plant lectin database LectinDB (Chandra *et al* 2006), the predicted PPA precursor possessed two domains, called PPA-DOM_1 and PPA-DOM_2. PPA-DOM_1 was between T_{27} and W_{130} amino acids, and PPA-DOM_2 was between D_{146} and S_{250}. The predicted PPA-DOM_1 molecular weight was 11.5 kDa with a pI of 5.6, while the predicted PPA-DOM_2 molecular weight was 11.7 kDa with a pI of 6.03. The amino acid sequences of PPA-DOM_1 and PPA-DOM_2 had 43% (47/107) identity. Comparison of the sequences of PPA-DOM_1 and PPA-DOM_2 with one-domain lectins such as GNA and AKA showed a higher identity.

Multiple mannose-binding lectins have been isolated and characterized. According to previous reports, lectins belonging to two-domain lectins contain three, two or one mannose-binding sites (Ramachandraiah and Chandra 2000). Until now, only ten two-domain lectins have been identified; these are ASA from Alliaceae (Van Damme *et al* 1992), HHA (AAD16404), CVA (AAG10404), CSA (AAK29007) from Iridaceae, THA (S62647) from Liliaceae, AMA (Van Damme *et al* 1995), CEA (Hirai *et al* 1993; Bezerra *et al* 1995), PTA (Yao *et al* 2003b), AHA (Lin *et al* 2005a) and ALA (Lin *et al* 2005b) from Araceae. These lectin precursors possess two domains, called DOM_1 and DOM_2. DOM_1 contains one mannose-binding site and DOM_2 contains two, one or no mannose-binding sites. For example, DOM_2 of ASA contains two mannose-binding sites. DOM_2 of AHA, PTA, ALA, CEA and AMA contains one mannose-binding site, while DOM_2 of THA, CVA, CSA and HHA contains no mannose-binding site (figure 2). These sites are essential for the mannose-binding property. The number of mannose-binding sites correlates with the capability for binding mannose. PPA precursors possessed two domains, DOM_1 containing one mannose-binding site and DOM_2, also containing one mannose-binding site. Apart from containing mannose-binding sites, some lectins contain an N-glycosylation site and an N-acetylgalactosamine-binding activity site. For example, ASA has two N-glycosylation sites, HTA one N-glycosylation site while AMA contains one N-acetylgalactosamine-binding activity site. The carbohydrate-binding characteristics of mannose-binding lectins differ markedly from other bulb lectins, some of which are able to bind simple sugars such as Glc, Man or Gal, while others have a much higher affinity for oligosaccharides. However, only mannose or mannose-rich oligosaccharides inhibit agglutination (Van Damme *et al* 1995). Native PPA can react with wheat germ agglutinin (WGA), Concanavalin A (ConA) and *Lens culinaris* agglutinin (LCA), but exhibit no reactivity toward *Phaseolus vulgaris* erythroagglutinin (PHA-E) (Sun *et al* 1995). The nature of the lectin correlates with its mannose-binding active site.

3.3 *Southern blot analysis*

The presence of multiple copies of the mannose-binding lectin gene in the genome has been reported in many plant species, particularly those belonging to the Amaryllidaceae

and Araceae families (Van Damme *et al* 1991, 1995; Hirai *et al* 1993). To investigate if *ppa* belonged to a multi-copy gene family, genomic DNA of *P. pedatisecta* was digested with *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII, respectively, followed by hybridization with the coding sequence of *ppa* as the probe. The result showed that multiple hybridization bands were present in each lane, indicating that *ppa* belonged to a multi-copy gene family (figure 3).

```
   1 CAGCCAGTAACACGGCGAACAAGTTATAAGAGTTTGTTAGGGTTTTGCACCTAGTAGCCA   60
  61 GGTAGCTAGCAGCAAGCCCCCTTCCTTCCTCATGGCCTCCAAGCTCCTCCTCTTCCTCCT  120
                                      M  A  S  K  L  L  L  F  L  L
 121 CCCGGCCATCCTCGGCCTCGTCATTCCTCGGCCAGCCGCGGCAGTGGGCACCAACTACCT  180
      P  A  I  L  G  L  V  I  P  R  P  A  A  A↓V  G  T  N  Y  L
 181 GCTGTCCGGCGAAACCCTAGACACGAACGGCCATCTCAGGAACGGCGACTTCGACTTGGT  240
      L  S  G  E  T  L  D  T  N  G  H  L  R  N  G  D  F  D  L  V
 241 CATGCAGGAGGACTGCAACGCCGTCCTGTACAACGGCAACTGGCAGTCCAACACGGCCAA  300
      M  Q  E  D  C  N  A  V  L  Y  N  G  N  W  Q  S  N  T  A  N
 301 CAAAGGACGGGACTGCAAGCTCACCCTCACCGACCGCGGCGAGCTCGTCATCAACAACGG  360
      K  G  R  D  C  K  L  T  L  T  D  R  G  E  L  V  I  N  N  G
 361 GGAGGGATCCATCGTCTTTAGGAGCGGCTCCCAGTCCGTGAGGGGCAACTACGCCTTGGT  420
      E  G  S  I  V  F  R  S  G  S  Q  S  V  R  G  N  Y  A  L  V
 421 CGTCCATCCGGAGGGGAGGCTAGTCATCTACGGCCCATCCGTCTTCAAGATCAACCCTTG  480
      V  H  P  E  G  R  L  V  I  Y  G  P  S  V  F  K  I  N  P  W
 481 GGTCCCCGGCCTCAACAGCCTGCGGCTCGGCAACATCCCTTTCACGGACAACATGCTCTT  540
      V  P  G  L  N  S  L  R  L  G  N  I  P  F  T  D  N  M  L  F
 541 CTCCGGCCAGGTCCTCTACGGCGACGGCAAGCTCACCGCGAGGAACCACATGCTCGTGAT  600
      S  G  Q  V  L  Y  G  D  G  K  L  T  A  R  N  H  M  L  V  M
 601 GCAGGGCGACTGCAACCTGGTCCTGTACGGCGGCAAGTACGGCTGGCAGTCCAACACCCA  660
      Q  G  D  C  N  L  V  L  Y  G  G  K  Y  G  W  Q  S  N  T  H
 661 CGGCAACGGCGAGCACTGCTTCGTGAGGCTGAGCCACAAGGGCGAGCTCATCATCAAGGA  720
      G  N  G  E  H  C  F  V  R  L  S  H  K  G  E  L  I  I  K  D
 721 CGACGACTTCCAGACCATCTGGAGCAGCCGATCCAGCTCCAAGCAGGGTGAGTACGTCTT  780
      D  D  F  Q  T  I  W  S  S  R  S  S  S  K  Q  G  E  Y  V  F
 781 CATCCTCCAGGACAACGGCTTCGGCGTCGTCTACGGCCCTGCCATTTGGGCGACCAGCTC  840
      I  L  Q  D  N  G  F  G  V  V  Y  G  P  A  I  W  A  T  S  S
 841 GAAGCGCTCCATTGCTGCG*TAG*TAGAAGGTGTCTTCCACCCCAGTGGAAAATAAGTGAGC  900
      K  R  S  I  A  A  *
 901 CCACGGACATAATCGTGCATGATCGACATGGTAGACTAAAATAAAGTTGGCGGCTATATA  960
 961 TATATATGCAATCGGTGCGGTTATTCCTTGTTGTAGTCTGTCTTCGTCCACAGTGTCTTG 1020
1021 TTAATAAGAGGCTTTGGTCTTTCCTTGCTTTCGTTGCCATATATAGTTCTGGCTTCTGCT 1080
1081 GCTGTATAAGTTTGTCCTTCGAATAATATGTATGGCTACCTTGATCAAAAAAAAAAAAAA 1140
1141 AAAAAA                                                        1146
```

Figure 1. cDNA sequence and deduced amino acid sequence of the *P. pedatisecta* agglutinin gene (*ppa*). The start codon (ATG) is in bold and the stop codon (TAG) in bold italics. Mannose-binding sites (QXDXN/LXVXY) are in boxes. The two polyadenylation signals are underlined. The predicted cleavage site (between A and V) of the signal peptide of PPA is indicated by an arrow.

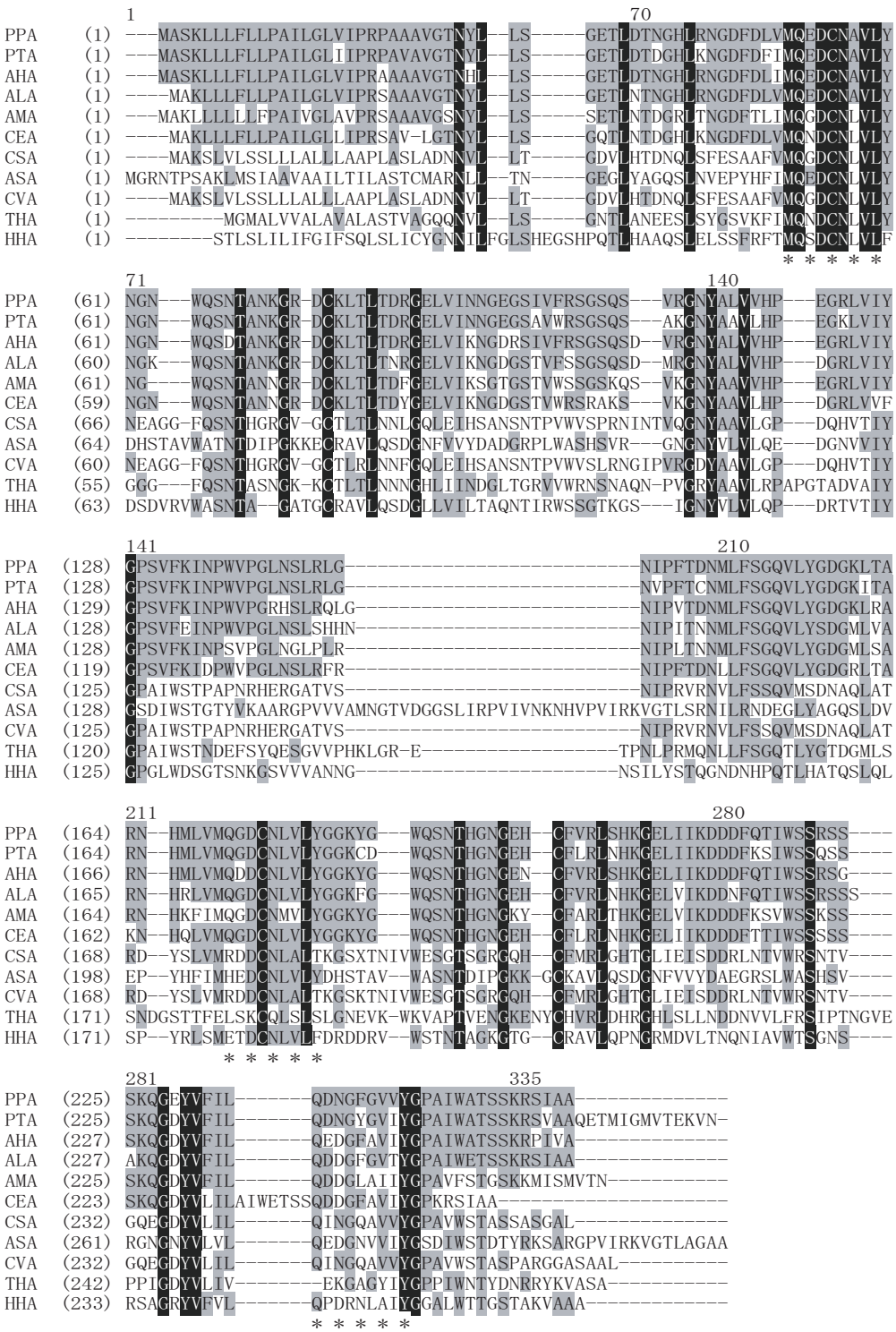

Figure 2. Multiple alignments of the amino acid sequence of PPA with those of other mannose-binding lectins. The alignment was performed with the Vector NTI Suite 8.0 by using the published mannose-binding lectin sequences of ALA (AAS66304), AHA (AAP50524), PTA (AAP20876), AMA (AAC48998), CEA (S56688), CSA (AAK29077), CVA (AAG10404), THA (S62647), ASA (AAB64239) and HHA (AAD16404). Gaps were introduced for optimal alignment and maximum similarity between all compared sequences. The identical amino acids among all the aligned sequences are shown on a black background and the identical amino acids with PPA are shown on a grey background. Mannose-binding sites are indicated with '*'.

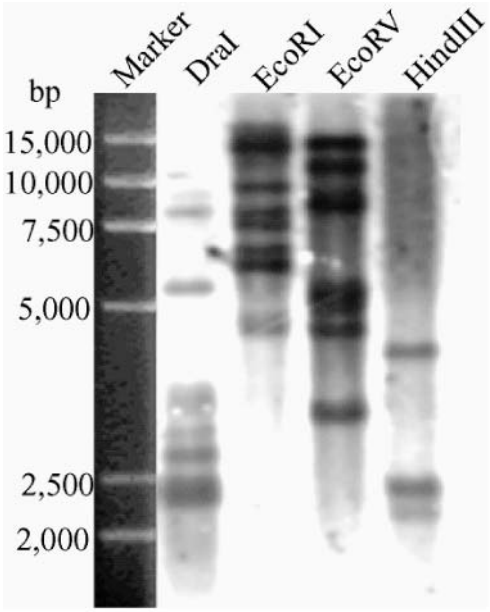

Figure 3. Southern blot analysis. Genomic DNA isolated from leaves of *P. pedatisecta* was digested with *Dra*I, *Eco*RI, *Eco*RV and *Hind*III, respectively, followed by hybridization with *ppa* coding sequence as the probe.

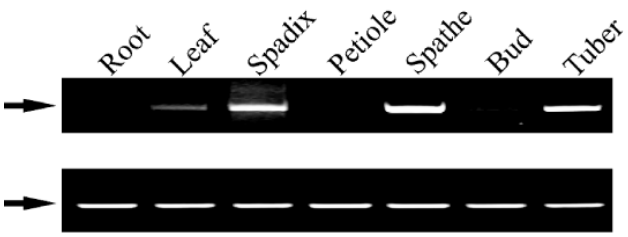

Figure 4. Expression profiling analysis of *ppa* in different tissues of *P. pedatisecta.* Total RNA was isolated separately from root, leaf, spadix, petiole, spathe, bulb and tuber of *P. pedatisecta* and subjected to semi-quantitative one-step RT-PCR analysis. Twenty cycles were used for the PCR amplification of *ppa* (upper panel) and 18S rRNA (lower panel, as the control).

3.4 *Expression profiling of ppa in various tissues*

To investigate the *ppa* expression pattern in various tissues of *P. pedatisecta*, total RNA was isolated from root, leaf, spadix, petiole, spathe, bud and tuber, respectively, and subjected to semi-quantitative one-step RT-PCR analysis using primers S1 and S3. The result showed that, like lectin genes from other Araceae species such as AHA (Zhao *et al* 2003), *ppa* expressed in most tissues but at various levels (figure 4). High expression was found in spadix, spathe and tuber, while low expression was found in leaf, petiole and bud, and almost no expression in root.

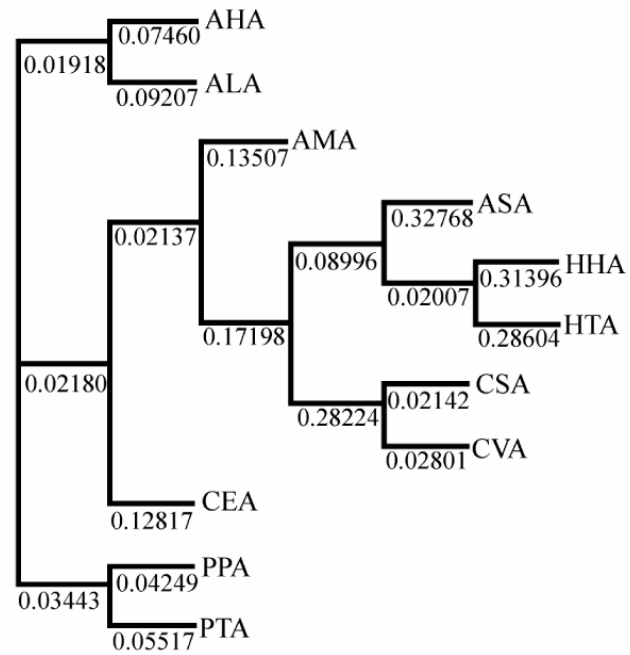

Figure 5. Dendrogram of mannose-binding lectins from Araceae, Iridaceae, Amaryllidaceae, Bromeliaceae and Orchidaceae families. A dendrogram tree was drawn using the CLUSTAL W program. The sequences of mannose-binding lectins used in the analysis were downloaded from NCBI, and the accession numbers were ALA (AAS66304), AHA (AAP50524), PTA (AAP20876), AMA (AAC48998), CEA (S56688), CSA (AAK29077), CVA (AAG10404), THA (S62647), ASA (AAB64239) and HHA (AAD16404).

In most species, mannose-binding lectins occur in almost all vegetative tissues, such as leaves, flowers, ovaries, bulbs, tubers, rhizomers, roots and nectar (Van Damme *et al* 1998). However, lectins from different tissues may differ in their isolectin composition. In some species, lectins from different tissues may differ in character. For example, ASA, the lectin from *Allium sativum*, contains two different bulb-specific lectins, one leaf-specific lectin, and one root-specific lectin (Van Damme *et al* 1992; Smeets *et al* 1997b). In other species, lectins from different tissues may differ in amino acid composition, such as GAFP-1, the antifungal protein from *Gastrodia elata*, which contains four mannose-binding lectins among which the amino acid sequence identity was 98.2% (Wang *et al* 2001). PPA contains some isoforms in different tissues and the results of this study show the pattern of *ppa* gene family expression.

3.5 *Dendrogram tree analysis*

The dendrogram tree analysis indicated that two-domain lectins from plants were divided into three groups

(figure 5). One group contained two members of mannose-binding lectins, AHA and ALA from *Arisaema* of Araceae (upper portion of figure 5), one group contained two mannose-binding lectins, PPA and PTA from *Pinellia* of Araceae (lower portion of figure 5), and the third group contained taxonomically unrelated mannose-binding lectins, including AMA from *Arum* of Araceae, CEA from *Colocasia* of Araceae, ASA from *Allium* of Alliaceae, CSA and CVA from *Crocus* of Iridaceae, THA from *Tulipa* of Liliaceae and HHA from *Hyacinthoides* of Hyacinthoides. All these three groups of lectins were derived from a common ancestor during evolution, suggesting that these lectins shared a common evolutionary ancestor in plants. It was also obvious that the dendrogram tree had genus-specific features, for example, two members of *Pinellia* and two members of *Arisaema* had a closer relationship with each other than with the others, and species of the same genus were clustered into one group. The results of the analysis highlighted that PPA was grouped into a cluster together with PTA from *Pinellia* of Araceae, indicating that PPA had a closer relationship with *Pinellia* of Araceae than with other genuses of Araceae.

We report on the cloning and characterization of the full-length cDNA of the lectin gene from *P. pedatisecta*. The cloning of *ppa* enables us to test whether it has a potential role in controlling pests and fungal diseases by transferring the gene into plants in the future.

Acknowledgements

This research was funded by China National Transgenic Plant Research and Commercialization Project, China National "863" High-Tech Program, China Ministry of Education and Shanghai Science and Technology Committee.

References

An J, Liu J Z, Wu C F, Li J, Dai L, Van Damme E J M, Balzarini J, De Clercq E, Chen F and Bao J K 2006 Anti-HIV I/II activity and molecular cloning of a novel mannose/sialic acid-binding lectin from rhizome of *Polygonatum cyrtonema* Hua; *Acta Biochim. Biophys. Sin.* **38** 70–78

Balzarini J, Hatse S, Vermeire K, Princen K, Aquaro S, Perno C F, De Clercq E, Egberink H, Vanden Mooter G, Peumans W, Van Damme E and Schols D 2004 Mannose-specific plant lectins from the Amaryllidaceae family qualify as efficient microbicides for prevention of human immunodeficiency virus infection; *Antimicrob. Agents Chemother.* **48** 3858–3870

Bezerra I C, Castro L A, Neshich G, de Almeida E R, de Sa M F, Mello L V and Monte-Neshich D C 1995 A corm-specific gene encodes tarin, a major globulin of taro (*Colocasia esculenta* L. Schott); *Plant Mol. Biol.* **28** 137–144

Chandra N R, Kumar N, Jeyakani J, Singh D D, Gowda S B and Prathima M N 2006 Lectindb: a plant lectin database; *Glycobiology* **16** 938–946.

Chen Z, Kai G, Liu X, Lin J, Sun X and Tang K 2005 cDNA cloning and characterization of a mannose-binding lectin from *Zingiber officinale* Roscoe (ginger) rhizomes; *J. Biosci.* **30** 213–220

Chen Z, Sun X and Tang K 2004 Genomic cloning and characterization of a novel lectin gene from *Zantedeschia aethiopica*; *Biosci. Rep.* **24** 225–234

Fei J, Liao Z, Chai Y, Pang Y, Yao J, Sun X and Tang K 2003 Molecular cloning and characterization of a novel mannose-binding lectin gene from *Amorphophallus konjac*; *Mol. Biol. Rep.* **30** 177–183

Hirai M, Nakamura K, Imai T and Sato T 1993 cDNAs encoding for storage proteins in the tubers of taro (*Colocasia esculenta* Schott); *Jpn. J. Genet.* **68** 229–236

Huang D, Pan Y, Zhang S, Cao J, Yang X, Zhang J and Yi W 1997 The discovery of insecticidal protein against aphids from *Pinellia pedatisecta* and *P. ternata*; *Sci. Agric. Sin.* **30** 94

Jaakola L, Pirttila A M, Halonen M and Hohtola A 2001 Isolation of high quality RNA from bilberry (*Vaccinium myrtillus* L.) fruit; *Mol. Biotechnol.* **19** 201–203

Kai G, Zhao L, Zheng J, Zhang L, Miao Z, Sun X and Tang K 2004 Isolation and characterization of a new mannose-binding lectin gene from *Taxus media*; *J. Biosci.* **29** 399–407

Lin J, Li Y, Zhou X, Tang K and Chen F 2003 Cloning and characterization of a curcin gene encoding a ribosome inactivating protein from *Jatropha curcas*; *DNA Seq.* **14** 311–317

Lin J, Liu J, Sun X, Zhou X, Fei J and Tang K 2005a An efficient method for rapid amplification of *Arisaema heterophyllum* agglutinin gene using a genomic walking technique; *Prep. Biochem. Biotechnol.* **35** 155–167

Lin J, Zhou X, Pang Y, Gao H, Fei J, Shen GA, Wang J, Li X Sun X and Tang K 2005b Cloning and characterization of an agglutinin gene from *Arisaema lobatum*; *Biosci. Rep.* **25** 345–362

Neuteboom L W, Kunimitsu W Y, Webb D and Christopher D A 2002 Characterization and tissue-regulated expression of genes involved in pineapple (*Ananas comosus* L.) root development; *Plant Sci.* **163** 1021–1035

Pan Y, Zhang S, Cao J, Yang X, Zhang J, Ying W and Huang D 1998 The isolation, purification of *Pinellia pedatisecta* lectin and its activity on aphid resistance; *Prog. Nat. Sci.* **8** 502–505

Ramachandraiah G and Chandra N R 2000 Sequence and structural determinants of mannose recognition; *Protein* **39** 358–364

Smeets K, Van Damme E J, Van Leuven F and Peumans W J 1997a Isolation, characterization and molecular cloning of a leaf-specific lectin from ramsons (*Allium ursinum* L.); *Plant Mol. Biol.* **35** 531–535

Smeets K, Van Damme E J, Verhaert P, Barre A, Rougé P, Van Leuven F and Peumans W J 1997b Isolation, characterization and molecular cloning of a mannose-binding lectin from leaves and roots of garlic (*Allium sativum* L.); *Plant Mol. Biol.* **33** 223–234

Sun G, Ding S and Qian Y 1992 The extraction and chemical analysis of proteins from *Pinellia pedatisecta* and their inhibitory effects on the mouse sarcoma-180; *Acta Acad. Med. Shanghai* **19** 17–20

Sun G, Ding S and Qian Y 1995 Purification and characterization of *Pinellia pedatisecta* lectin A; *Acta Acad. Med. Shanghai* **22** 299–302

Tao Z, Xu Q, Wu K, Lian S and Sun D 1981 Isolation, crystallization, biological activities and some chemical characteristics of pinellin; *Acta Biochim. Biophys. Sin.* **13** 77–82

Van Damme E J, Astoul C H, Barre A, Rougé P and Peumans W J 2000 Cloning and characterization of a monocot mannose-binding lectin from *Crocus vernus* (family Iridaceae); *Eur. J. Biochem.* **267** 5067–5077

Van Damme E J, Briké F, Winter H C, Van Leuven F, Goldstein I J and Peumans W J 1996 Molecular cloning of two different mannose-binding lectins from tulip bulbs; *Eur. J. Biochem.* **236** 419–427

Van Damme E J, Goossens K, Smeets K, Van Leuven F, Verhaert P and Peumans W J 1995 The major tuber storage protein of Araceae species is a lectin: Characterization and molecular cloning of the lectin from *Arum maculatum* L.; *Plant Physiol.* **107** 1147–1158

Van Damme E J, Kaku H, Perini F, Goldstein I J, Peeters B, Yaqi F, Decock B and Peumans W J 1991 Biosynthesis, primary structure and molecular cloning of snowdrop (*Galanthus nivalis* L.) lectin; *Eur. J. Biochem.* **202** 23–30

Van Damme E J, Peumans W J, Barre A and Rougé P 1998 Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles; *Crit. Rev. Plant Sci.* **17** 575–692

Van Damme E J, Smeets K, Torrekens S, van Leuven F and Peumans W J 1993 The mannose-specific lectins from ramsons (*Allium ursinum* L.) are encoded by three sets of genes; *Eur. J. Biochem.* **217** 123–129

Van Damme E J, Smeets K, Torrekens S, van Leuven F, Goldstein I J and Peumans W J 1992 The closely related homomeric and heterodimeric mannose-binding lectins from garlic are encoded by one-domain and two-domain lectin genes, respectively; *Eur. J. Biochem.* **206** 413–420

von Heijne G 1986 A new method for predicting signal sequence cleavage sites; *Nucleic Acids Res.* **14** 4683–4690

Wang X C, Bauw G, Van Damme E J, Peumans W J, Chen Z L, Montagu M V, Angenon1G and Dillen W 2001 Gastrodianin-like mannose-binding proteins: a novel class of plant proteins with antifungal properties; *Plant J.* **25** 651–661

Wright L M, Van Damme E J, Barre A, Allen A K, van Leuven F, Reynolds C D, Rougé P and Peumans W J 1999 Isolation, characterization, molecular cloning and molecular modelling of two lectins of different specificities from bluebell (*Scilla campanulata*) bulbs; *Biochem. J.* **340** 299–308

Yao J, Pang Y, Qi H, Wan B, Zhao X, Kong W, Sun X and Tang K 2003 Transgenic tobacco expressing *Pinellia ternata* agglutinin confers enhanced resistance to aphids; *Transgenic Res.* **12** 715–722

Zhao X, Yao J, Liao Z, Zhang H, Chen F, Lu Y, Song J, Sun X, Yu S and Tang K 2003 Molecular cloning of a novel mannose-binding lectin gene from *Arisaema heterophyllum; Plant Sci.* **165** 55–60

Zhu M, Zhou K and Ding S 1999 Total proteins of *Pinellia pedatisecta* effects in ovarian cancer cell lines and human umbilical cord blood hematopoietic progenitors; *J. Shanghai Med. Univ.* **26** 455–458

MS received 20 June 2006; accepted 8 November 2006

*e*Publication: 3 January 2007

Corresponding editor: Vidyanand Nanjundiah