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Full Length Research Paper

Molecular cloning and characterization of a pathogen-related protein PR10 gene in pyrethrum (*Chrysanthemum cinerariaefolium*) flower response to insect herbivore

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In this study we successfully constructed a full-length cDNA library from open flower of pyrethrum (*Chrysanthemum cinerariaefolium*), the most well-known natural source of insecticide. By random sequencing, a gene coding pathogen-related (PR) protein PR10 was identified and characterized. It was designated as *CcPR10*, encoding a protein of 157 amino acids. The calculated molecular weight of the *CcPR10* protein was 17.2 kDa and the theoretical isoelectric point was 5.98. Sequence analysis showed that the *CcPR10* protein was highly homologous with other PR10 proteins. The expression of *CcPR10* gene was examined in various tissues of mature plant by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and the result show that the expression of *CcPR10* was observed in flowers and roots. The expression of *CcPR10* gene was induced by wounding and jasmonic acid treatments. Moreover, *CcPR10* transcript was induced by armyworm (*Spodoptera litura*) feeding, which suggests that *CcPR10* may be involved in pyrethrum resistance to insect herbivore.

Key words: Pathogen-related, protein PR10, Pyrethrum (*Chrysanthemum cinerariaefolium*), cDNA library, gene cloning.

INTRODUCTION

Most plants have the ability to protect themselves against abiotic and biotic stresses by synthesis and/or accumulation of a number of proteins called ‘‘pathogen-related’’ (PR) protein (Dixon and Lamb, 1990). PR proteins can be grouped into 17 independent classifications, PR-1 to PR-17, based on their amino acid sequence, enzymatic and biological activities (Van Loon and Van Strien, 1999). The PR10 family is identified in a number of plant species, such as pea (Barral and Clark, 1991), asparagus (Warner et al., 1992), soybean (Crowell et al., 1992), bean (Walter et al., 1996), sorghum (Lo et al., 1999), barley (Steiner-Lange et al., 2003), rice (Hashimoto et al., 2004) and plum (EI-kereamy et al., 2009).

PR10 proteins have been identified as ribonuclease-like PR proteins base on amino acid sequence similarity (Van loon et al., 1994). Two PR10 proteins from ginseng were first cloned as plant ribonucleases (Moiseyev et al., 1997). The ribonucleolytic activity was found in major birch-pollen allergen, Bet v 1, which was known to have homology to PR10 proteins (Bufe et al., 1996). Bantignies et al. (2000) demonstrated that a PR10-like protein from white lupine (*Lupinus albus*) roots had ribonucleolytic activity. It was reported that the induction and subsequent phosphorylation of CaPR10 increased its ribonucleolytic activity to cleave invading viral RNAs (Park et al., 2004).

Moreover, Liu and Ekramoddoullah (2006) reported that some PR10 proteins have shown antifungal or

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Abbreviations: PR, Pathogen-related; **JA,** jasmonic acid.

antibacterial activity and the level of such activities varied among species of the target microorganism. Induction of *PR10* expression has been demonstrated in a wide variety of plant species following infection by pathogens and abiotic stresses (Borsics and Lados, 2002; Agrawal et al., 2002; Zhang et al., 2010). Expression of some PR10 genes is also up-regulated following treatment with plant hormones (Rakwal et al., 1999; Borsics and Lados, 2002; Poupard et al., 2003).

Pyrethrum, a perennial herbaceous plant of the Compositae, is economically important as a natural source of insecticide. Pyrethrin, derived from the pyrethrum flower, serves as high effective, widely used, low toxic and pollution-free pesticide. In this study, we successfully constructed a full-length cDNA library from open flower of pyrethrum. This cDNA library will be helpful to find the candidate genes involving in pyrethrin biosynthesis and understand the mechanism of insect tolerance of pyrethrum plant.

A gene encoding putative PR10 protein in pyrethrum was isolated and characterized. CcPR10 was highly homologous with PR proteins. The expression of *CcPfR10* gene was induced by wounding and jasmonic acid (JA) treatments in immature leaves. Moreover, *CcPR10* transcript was significantly induced by armyworm (*Spodoptera litura*) feeding. Based on these data, we suppose that *CcPR10* may be involved in pyrethrum resistance to insect herbivore.

MATERIALS AND METHODS

Isolation and purification of total RNA

Total RNA was isolated from flower of pyrethrum by using WATSON RNA isolation kit according to the manufacturer's instructions (Watson Biotechnologies, Shanghai, China). Then the total RNA was digested by DNase I to clear out remnant DNA and its concentration and quantity were determined by detection of optical density (OD) value and electrophoresis.

Construction of the cDNA library

A cDNA expression library was constructed from open flower of pyrethrum (all disc florets open), using Creator™ SMART™ cDNA Library Construction Kit according to the manufacturer's instructions (Clontech, Palo Alto, CA, USA). 1 µg RNA was combined with 1 µl of SMART IV oligonucleotide (Clontech) and 1 µl CDS-3M adapter for first-strand cDNA synthesis. The first strand cDNA was initially amplified by long-distance PCR (LD-PCR) using hot-start amplification. Cycle settings were 95°C for 3 min followed by 23 cycles of 95°C for 10 s, 66°C for 20 s, and 72°C for 4 min. The product was analyzed on a 1.1% agarose gel to determine the sizes and amount of the cDNA products before proceeding to the next step. The LD-PCR reaction was purified and eluted in 30 µl of sterile water using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). The cDNA from the LD-PCR was normalized by duplex-specific nuclease (DSN) enzyme (Clontech) for 20 min at 70°C. The DSN enzyme specifically degrades double-stranded molecules. Following normalization, one round of PCR was performed with primer M1 (first 23 bases of the SMART IV oligonucleotide) and 1 µl of the normalization reaction as template. Cycle settings were 95°C for 3 min followed by 20 cycles of 95°C for 10 s, 66°C for 20 s, and 72°C for 4 min. Products were checked on a 1.1% agarose gel. The PCR products were purified and digested with restriction endonuclease *Sfi* I. Then, the cDNA was ligated to the pDNR-LIB vector and the ligation production was transformed into *Escherichia coli* JM109 by electroporation. The library was tittered, amplified, and stored in glycerol stocks in a -80°C freezer.

Library sequencing and analysis

The cDNA library clones were plated into lysogeny broth (LB) agar plate and clones were picked randomly. Plasmids DNA of each clone were prepared by standard alkaline lysis preparation protocol and then sequenced by Shanghai Sangon Biological Engineering Company. The sequences were subjected to blast analysis against the non-redundant database on the Genbank (http://www.ncbi.nlm.nih.gov/blast).

Sequence analysis

The DNA sequences were analyzed with DNAstar software. Domain structure of protein was searched for with the Pfam program (http://pfam.wustl.edu/) and the BLASTP program. Protein sequences were aligned with the ClustalX version 2.0. Phylogenetic and molecular evolutionary analyses were constructed by maximum parsimony (MP) method using MEGA version 4 (Tamura et al., 2007). The reliability of the tree was measured by bootstrap analysis with 1000 replicates (Felsenstein et al., 1985). The isoelectric point was determined by DNAstar program.

Plant treatments and expression analysis

For determining tissue specific expression, seven-month-old, untreated plants were dissected to obtain material of each of the described tissues. Wounding-, insect-, and JA-treatments were done using five-week-old pyrenthrum plants. Wounding was inflicted by crushing leaflets several times with a hemostat. JA was made up in 1% dimethylysulfoxide (DMSO) as a stock solution at 100 mM, and used at concentrations described in the legends to figures. Control treatments involved addition of equivalent amount of 1% DMSO in distilled water without JA. Four armyworms were put on one plant. Wounding, hormone-treatments, and armyworm-feeding were done at 0 h and leaves were sampled at 4 and 10 h. RNA was extracted using RNA extraction kit (Promega, Madison, WI, USA). DNase-treated RNA (2 mg) was then reverse-transcribed in a total volume of 20 µl using the reverse transcription kit (TakaRa, Dalian, China). For semi-quantitative RT-PCR, the pyrethrum 18S was used as constitutively expressed control gene. The 18S forward primer was 5'-CTCATGGGATGTGGCTTCTT-3' and 18S reverse primer was 5'-GCGTTCAAAAACTCGATGGT-3'. Initial PCR was performed with 18S primers, product levels were compared and individual cDNA samples were diluted accordingly, to ensure equal template concentrations. PCRs with primers of *CcPR10* gene were subsequently performed for non-saturating number of amplification cycles. The *CcPR10* gene forward primer was 5'-GGTCTTCACTTCCCGCTGAT-3' and reverse primer was 5'-TCGTGGGCCTTGAAGGTGTT-3'. PCR conditions were as follow: 95°C for 5 min, then 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and finally 72°C for 10 min. 18S primers were always included in each experiment. The PCR products were separated on 0.9% agarose gel and semi-quantified with Band leader Application Version 3.0. The fold induction of *CcPR10* mRNA level normalized

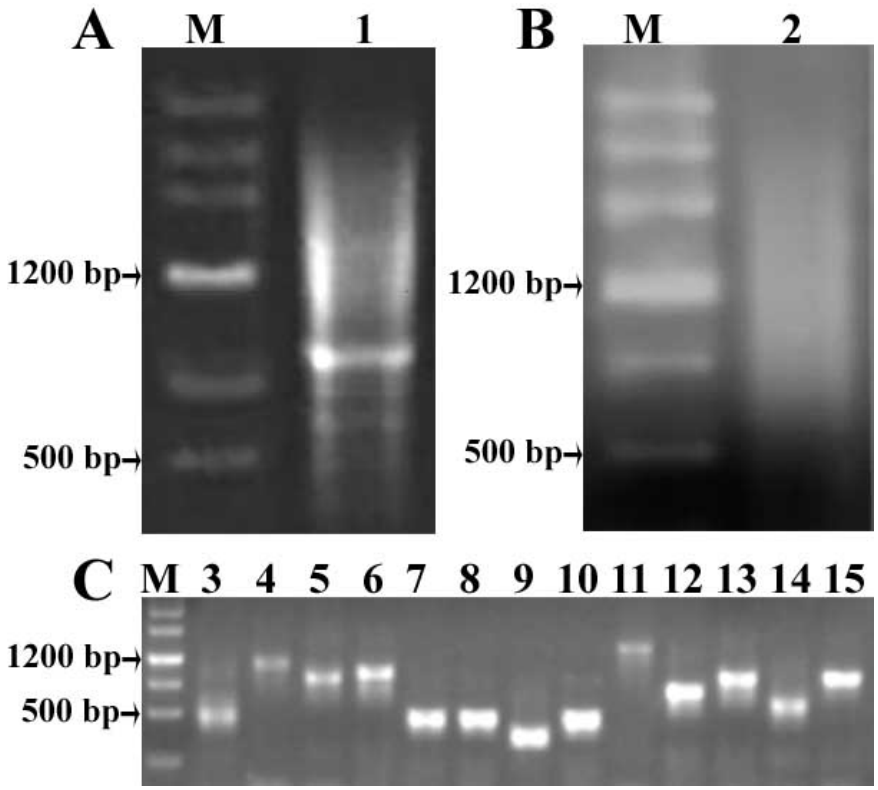

Figure 1. Agarose gel electrophoresis of the synthesized double-strand cDNA (A), DSN-normalized cDNA (B), and the PCR products of insertion fragments from the clones selected randomly (C). Lane 1, double-strand cDNA. Lane 2, DSN-normalized cDNA. Lanes 3-15 show the PCR products of different clones. Lane M, DNA size marker.

with the level of 18S gene, and the value stood for an n-fold difference relative to the blank. The results were given as mean ± S.E. (n = 3).

RESULTS AND DISCUSSION

Construction of cDNA library from open flower of pyrethrum

The flowers were used to extract total RNA. One microgram of total RNA was subjected to reverse transcription for synthesis of the first and double-strand cDNAs. Double-strand cDNA was concentrated on range of 2 000 to 500 bp (Figures 1A and B), suggesting that double-strand cDNAs were successfully synthesized. Agarose gel electrophoresis showed that bands corresponding to abundant transcripts, which were clearly visible in the pattern of non-normalized cDNA (Figure 1A), were reduced in the DSN-normalized cDNA sample (Figure 1B). The average cDNA length remains unchanged after the DSN-normalization. Normalization is utilized to enhance the gene discovery rate of a cDNA library and facilitate the identification and analysis of rare transcripts. This approach is imperative for functional screening, construction of specific RNA libraries, EST sequencing of the complete transcriptome, and transcript end sequence profiling. The capacity of the unamplified constructed cDNA library was 2.8×10^5 pfu/ml after calculation of clone numbers, which should meet almost all requirements to find a cDNA derived from a low-abundance mRNA. In order to assess the recombination rate of the unamplified cDNA library, 160 clones were picked randomly and sequenced. 153 clones (96%) contain vectors with inserted cDNA fragments and seven clones (4%) contain empty vectors. This result indicates that the recombination rate of the unamplified cDNA library is 96%. For better preserving, the cDNA library was amplified, resulting at least 2.0×10^9 pfu/ml. Then 50 clones were picked randomly to perform the colony PCR for confirming the size of inserted fragments within recombination plasmids. The amplified cDNA fragments ranged from 500 to 2 000 bp (Figure 1C), suggesting that the insertion fragments harbored most of the mRNAs and reached the requirement for further studies on gene structure, translation, and expression (Sambrook and Russell, 2001). Thus, we successfully constructed a full-length cDNA library from pyrethrum flower with high quality, providing a useful resource to find the candidate genes involving in

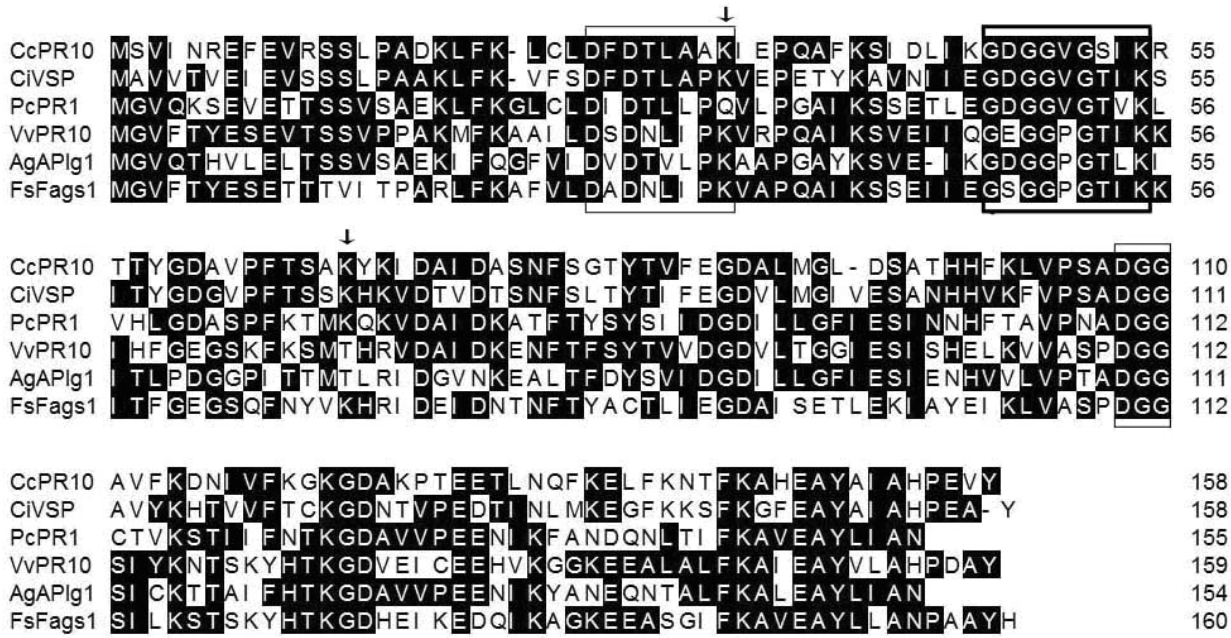

Figure 2. Sequence alignment of CcPR10 with five related proteins including allergens and PR proteins. Conserved residues are shaded in black and conserved motifs are framed. Arrows indicate the localization of the two lysine residues flanking the P-loop motif (bold frame). Genbank accession number: CcPR10 (JN211117), CiVSP (CAB71301), PcPR1 (CAA67246), VvPR10 (CAC16166), AgAP1g1 (CAA88831), FsFags1 (ACJ23866).

pyrethrin biosynthesis and understand the mechanism of insect tolerance of pyrethrum plant.

Cloning and sequence analysis of *CcPR10*

Among the 153 recombinant clones, one cDNA fragment has a 5' untranslated region (UTR) of 60 bp, a 3' UTR of 193 bp with a canonical polyadenylation signal sequence and a poly (A) tail, and an open reading frame (ORF) of 474 bp encoding a polypeptide of 157 amino acids. The predicted molecular weight of this protein was 17.2 kDa and isolectric point was 5.98. Based on the deduced amino acid sequence, this protein is homologous to a group of proteins belonging to the PR10 family (Figure 2) (Moiseyev et al., 1994; Xu et al., 2000). We therefore referred to this protein as CcPR10. PR10 family is defined by common structural properties and shares no similarity with others families of PR proteins (Walter et al., 1996). This protein family includes intracellular proteins induced by pathological or related stresses, and some allergens structurally related to PR10 proteins. CcPR10 and PR10 proteins have in common an acidic isoelectric point, a highly conserved low molecular weight (154 to 160 amino acids, 16 to 18 kDa), a highly conserved sequence motifs representative of PR10 protein family (Walter et al., 1996). Moreover, CcPR10 and PR10 proteins share the Kx13GxGGxGxxKx13K motif known as 'P-loop' motif which is implicated in phosphate binding (Breiteneder et al., 1989; Hoffmann-Sommergruber et al., 1997) and they both lack signal sequences targeting to the extracellular space or the vacuole (Somssich et al., 1988; Walter et al., 1990).

The deduced *CcPR10* was aligned with CiVSP, *Petroselinum crispum* PcPR1, *Vitis vinifera* VvPR10, *Apium graveolens* AgAP1g1, *Fagus sylvatica* FsFags1 by using Clastal X package of DNAstar program. As shown in Figure 3, CcPR10 shared homologies with the PcPR1 (45% identity) and VvPR10 (44%). PcPR1 is a typical member of the PR10 family (Sels et al., 2008). Homologies were also noticed with a group of allergens from the *F. sylvatica* (FsFagF1, 39% identity) and from celery (AgAPIg1, 39% identity).

In addition to the homology degree, the sequences of all these proteins were similar in size and no gaps were needed to maximize the alignment. Furthermore, the proteins shared several common motifs, identified by boxes on Figure 2. The motifs DxDxLxPK, GxGGxGT and DGG are putative allergen epitopes (Breiteneder et al., 1989) and the $Kx_{13}GxGGxGxxKx_{13}K$ motif is known as P-loop motif, facilitating binding of ribose and phosphate (Hoffmann-Sommergruber et al., 1997). Phylogenetic analysis was conducted to assess the relationships between CcPR10 and other PR proteins and allergen proteins. The phylogenetic tree was divided into two subgroups. One subgroup contains PR proteins, allergen protein and CiVSP and another subgroup contains OsPR10 (Figure 3). CcPR10 was grouped into the first subgroup and closely related to the PR10 proteins.

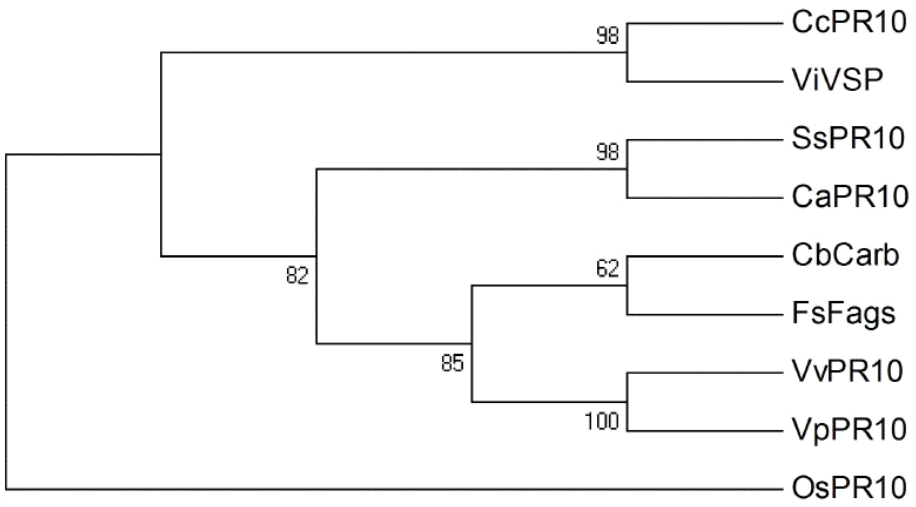

Figure 3. The phylogenetic relationship of the CcPR10 with PR protein, VSP protein, and allergen proteins. This tree is setup with the distance matrix using the maximum parsimony method. Poisson correction with the complete deletion of gaps was used to calculate protein distances. Bootstrap values (1000 bootstrap samples) are shown beside the branches. Genbank accession number: SsPR10 (AAU00066), CaPR10 (AAF63519), CbCarb (CAB02208), VvPR10 (CAC16166), VpPR10 (ABC86747), OsPR10 (AAL74406).

Expression of *CcPR10* gene

Semi-quantitative RT-PCR was performed to analyze the expression pattern of *CcPR10* gene in different tissues of mature pyrethrum plant. The results show that *CcPR10* gene was expressed in a tissue-specific manner in mature plant. There was high expression level in early flower and open flower and low expression in root, whereas almost no expression was detected in bud, shoot and leaf (Figure 4A). These results agree with expression of *CsPR10* from *Crocus sativus* which shows *CsPR10* is expressed in flower tissues and roots, not expressed in leaves (Gómez-Gómez et al., 2011). Moreover, the expression pattern of *CcPR10* gene was also analyzed under the treatment of wounding and JA. We used the leaves of immature plants (five-weeks-old) for treatment, in which the expression of *CcPR10* gene could be detected by semi-quantitative RT-PCR. The wounding treatment caused an obvious increase in wounded leaves after 4 h, but decrease after 10 h (Figure 4B). The *CcPR10* mRNA levels were increased in leaves within 4 h after the treatment of JA and continued to maintain for at least 10 h. Interesting, the expression of *CcPR10* was not induced within 4 h, but induced obviously at 10 h in the untreated leaves (Figure 4C). Moreover, we analyzed the expression of *CcPR10* gene in the immature plants in susceptibility to armyworm. The expression of *CcPR10* was induced by armyworm-feeding in immature leaves (Figure 4D). This data supports the hypothesis that *CcPR10* may involved in pyrethrum resistance to insect herbivore.

Many PR10 genes are induced by wounding, such as *PR10a* in potato (Constabel and Brisson, 1995), *PR10a* and *PR10c* in birch (Poupard et al., 1998), *PmPR10* in western white pine (Liu et al., 2005), and *MsPR10* in alfalfa (Borsics and Lados, 2002). Our results also display that the wounding treatment caused an obvious increase of *CcPR10* expression. Many PR10 genes were up-regulated when plants were exposed to biotic stresses, such as viruses (Xu et al., 2003), bacteria (Robert et al., 2001) or fungi (Jwa et al., 2001). Further studies found that some PR10 proteins showed antimicrobial activities *in vitro* against bacteria, fungi and viruses, such as Ocatin (Flores et al., 2002), SsPR10 (Liu et al., 2006) and CaPR10 (Park et al., 2004). SsPR10 and CaPR10 have shown antifungal or antibacterial activity and the level of such activities varied among species of the target microorganism (Park et al., 2004; Liu et al., 2006). CaPR10 can inhibit hyphal growth of *Phytophthora capsici* (Park et al., 2004). The recombinant protein SsPR10 inhibits the hyphal growth of *Pyricularia oryzae*, but do not inhibit the hyphal growth of *Sclerotinia sclerotiorum* and *Gibberella zeae* (Liu et al., 2006). Recent research shows that CpPRI, a PR-10 protein purified from *Crotalaria pallida* roots, exhibits papain inhibitory activity against root-knot nematodes, which are the most widespread pest (Andrade et al., 2010). Our results show that the expression of *CcPR10* is induced by armyworm-feeding and we suppose that *CcPR10* may be involved in pyrethrum resistance to insect herbivore.

In the present study, it was found that *CcPR10* gene was rapidly induced by JA treatment. The lily *PR10* genes are induced by methyl jasmonate (MeJA) in the anther and various other organs of lily plants (Wang et al., 1999). In rice, OsPR10 mRNA accumulated rapidly

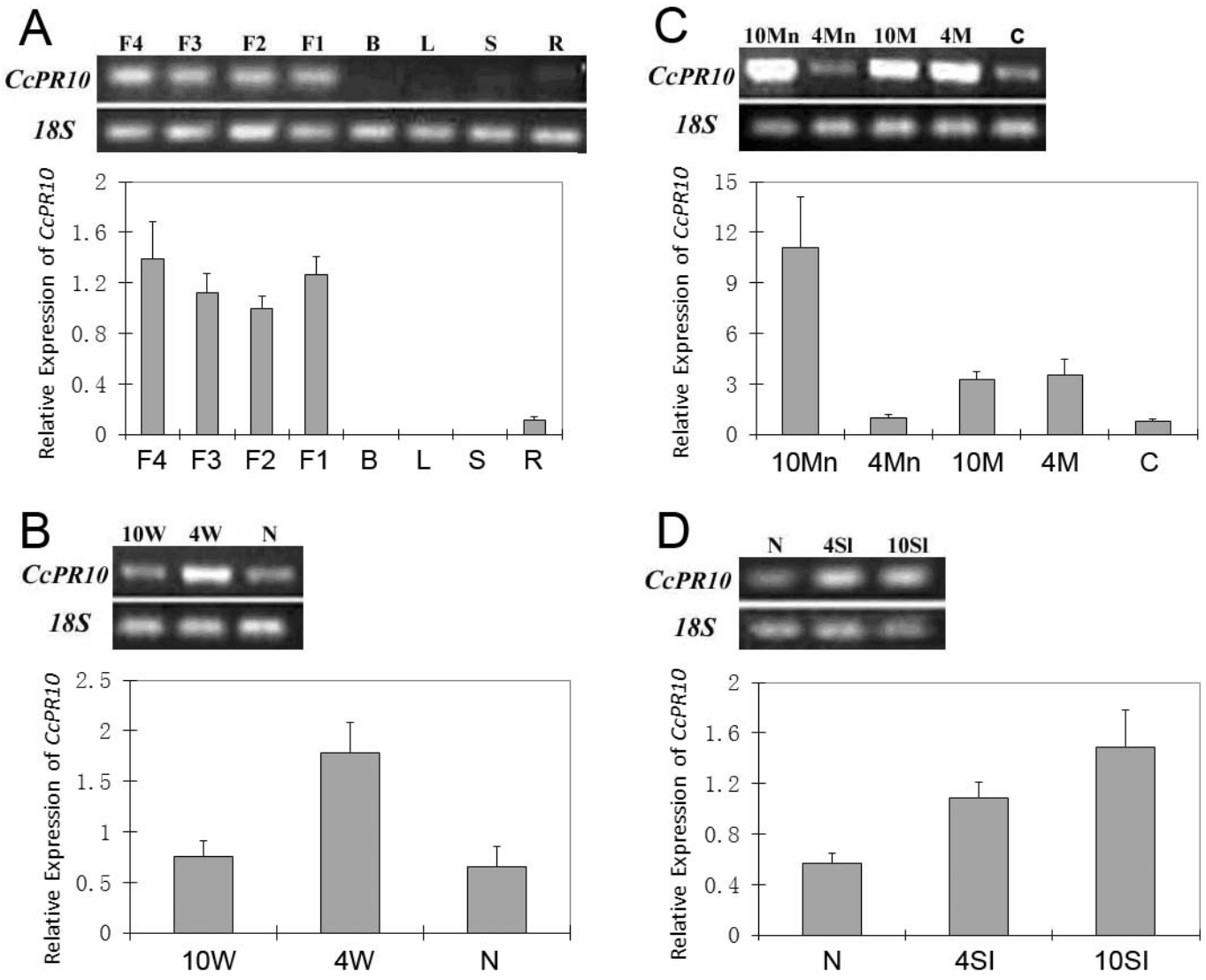

Figure 4. Semi-quantitative RT-PCR analysis of expression of *CcPR10* gene. Semi-quantitative analysis of the *CcPR10* gene transcript relative to 18S transcript. A, Expression of *CcPR10* in different tissues; R, root; S, shoot; L, leaf; B, bud; F1, early flower (first row of disc florets open); F2, open flower (all disc florets open); F3, ray florets from open flower; F4, disc florets from open flower. B, Expression of *CcPR10* in response to wounding; N, untreated leaves at 0 h; 4W, treated leaves at 4 h after wounding; 10W, treated leaves at 10 h after wounding. C, Expression of *CcPR10* in response to jasmonic acid (10 μM). 4M, treated leaves at 4 h after jasmonic acid spray; 10M, treated leaves at 10 h after jasmonic acid spray; 4Mn, untreated leaves at 4 h after jasmonic acid spray; 10Mn, untreated leaves at 10 h after jasmonic acid spray; C, control plants. D, Expression of *CcPR10* in response to armyworm-feeding. N, untreated leaves at 0 h; 4SI, treated leaves at 4 h after armyworm feeding; 10SI, treated leaves at 10 h after armyworm feeding. The results are given as mean ± S.E. (n = 3).

upon JA (Hwang et al., 2008; Hashimoto et al., 2004). In cotton suspension cells, when JA was added, a clear induction of *PR10* gene was indeed observed (Zhou et al., 2002). Expression of *CsPR10* from *C. sativus* was induced by JA in stigma tissues and *CsPR10* is induced through activation of the JA signaling pathway (Gómez-Gómez et al., 2011). JA and its biologically active derivatives have a critical role in regulating plant response to wounding or insect herbivore (Wasternack et al., 2007). It is found that treatment of plants with JA results in reprogramming of gene transcription, expression of defensive traits, and increased resistance to insect herbivores (Kessler and Baldwin, 2002; Mandaokar et al., 2006; Howe and Jander, 2008). Many JA-responsive genes are induced by herbivory, and proteins encoded by many of these genes have a confirmed role in anti-insect defense. In this study, *CcPR10* is induced by wounding, insect feeding and JA treatment. These results may suggest that *CcPR10* is induced possibly through activation of the JA signaling pathway.

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REFERENCE

Agrawal GK, Rakwal R, Tamogami S, Yonekura M, Kubo A, Saji H (2002). Chitosan activates defense/stress response(s) in the leaves of Oryza sativa seedlings. Plant Physiol. Biochem. 40: 1061-1069.

Andrade LB, Oliveira AS, Ribeiro JK, Kiyota S, Vasconcelos IM, de Oliveira JT, de Sales MP (2010) Effects of a novel pathogenesis-related class 10 (PR-10) protein from *Crotalaria pallida* Roots with papain inhibitory activity against root-knot nematode *Meloidogyne incognita*. J. Agric. Food Chem. 58: 4145-4152.

Bantignies B, Séguin J, Muzac IM, Dédaldéchamp F, Gulick P, Ibrahim R (2000). Direct evidence for ribonucleolytic activity of a PR-10-like protein from white lupin roots. Plant Mol. Biol. 42: 871-881.

Barral DHP, Clark JA (1991). Proteins arising during the late stages of embryogenesis in *Pisum sativum*. Planta, 184: 14-23.

Borsics T, Lados M (2002). Dodder infection induces the expression of a pathogenesis-related gene of the family PR-10 in alfalfa. J. Exp. Bot. 53: 1831-1832.

Breiteneder H, Petteburger K, Bito A, Valenta R, Kraft D, Rumpold H, Scheiner O, Breitenbach M (1989). The gene coding for the major birch pollen allergen BetvI, is highly homologous to a pea disease resistance response gene. EMBO J. 8: 1935-1938.

Bufe A, Spangfort MD, Kahlert H, Schlaak M, Becker WM (1996). The major birch pollen allergen, Bet v1, shows ribonuclease activity. Planta, 199: 413-415.

Constabel CP, Brisson N (1995). Stigma- and vascular-specific expression of the PR10a gene of potato, a novel pattern of expression of a pathogenesis-related gene. Mol. Plant Microbe Interact. 8: 104-113.

Crowell DN, John ME, Russell D, Amasino RM (1992). Characterization of a stress-induced, developmentally regulated gene family from soybean. Plant Mol. Biol. 18: 459-466.

Dixon RA, Lamb CJ (1990). Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 339-367.

El-kereamy A, Jayasankar S, Taheri A, Errampalli D, Paliyath G (2009). Expression analysis of a plum pathogenesis related 10 (PR10) protein during brown rot infection. Plant Cell Rep. 28: 95-102.

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap Evolution. 39: 783-791.

Flores T, Alape-Girón A, Flores-DÍaz M, Flores HE. (2002) Ocatin, a novel tuber storage protein from the andean tuber crop oca with antibacterial and antifungal activities. Plant Physiol. 128: 1291-302.

Gómez-Gómez L, Rubio-Moraga A, Ahrazem O (2011) Molecular cloning and characterisation of a pathogenesis-related protein CsPR10 from *Crocus sativus*. Plant Biol. 13: 297-303.

Hashimoto M, Kisseleva L, Sawa S, Furukawa T, Komatsu S, Koshiba T (2004). A novel rice PR10 protein, RSJIOsPR10, specifically induced in roots by biotic and abiotic stresses, possibly via the jasmonic acid signaling pathway. Plant Cell Physiol. 45: 550-559.

Hoffmann-Sommergruber K, Vanek-Krebitz M, Radauer C, Wen J, Ferreira F, Scheiner O, Breiteneder H (1997). Genomic characterization of members of the Bet v1 family: genes coding for allergens and pathogenesis-related proteins share intron positions. Gene, 197: 91-100.

Howe G, Jander G (2008). Plant immunity to insect herbivores. Annu. Rev. Plant. Biol. 59: 41-66.

Hwang SH, Lee IA, Yie SW, Hwang DJ (2008). Identification of an OsPR10a promoter region responsive to salicylic acid. Planta, 227: 1141-1150.

Jwa NS, Agrawal GK, Rakwal R, Park CH, Agrawal VP (2001) Molecular cloning and characterization of a novel jasmonate inducible pathogenesis-related class10 protein gene, JIOsPR10, from rice (*Oryza sativa* L.) seedling leaves. Biochem. Biophys. Res. Commun. 286: 973-83.

Kessler A, Baldwin IT (2002). Plant responses to insect herbivory: the emerging molecular analysis. Annu. Rev. Plant Biol. 53: 299-328.

Liu JJ, Ekramoddoullah AK, Piggott N, Zamani A (2005). Molecular cloning of a pathogen/wound-inducible PR10 promoter from *Pinus monticola* and characterization in transgenic Arabidopsis plants. Planta, 221: 159-169.

Liu JJ, Ekramoddoullah AKM (2006). The family 10 of plant pathogenesisrelated proteins: their structure, regulation, and function in response to biotic and abiotic stresses. Physiol. Mol. Plant Pathol. 68: 3-13.

Liu X, Huang B, Lin J, Fei J, Chen Z, Pang Y, Sun X, Tang K (2006). A novel pathogenesis-related protein (SsPR10) from *Solanum surattense* with ribonucleolytic and antimicrobial activity is stress- and pathogen-inducible. J. Plant Physiol. 163: 546-556.

Lo SC, Hipskind JD, Nicholson RL (1999). cDNA cloning of a sorghum pathogenesis-related protein (PR-10) and differential expression of defense-related genes following inoculation with *Cochliobolus heterostrophus* or *Colletotrichum sublineolum*. Mol. Plant Microbe Interact. 12: 479-489.

Mandaokar A, Thines B, Shin B, M.Lange B, Choi G, Koo YJ, Yoo YJ, Choi YD, Choi G, Browse J (2006). Transcriptional regulators of stamen development in Arabidopsis identified by transcriptional profiling. Plant J. 46: 984-1008.

Moiseyev GP, Beintema JJ, Fedoreyeva LI, Yakovlev GI (1994). High sequence similarity between a ribonuclease from ginseng calluses and fungus-elicited proteins from parsley indicates that intracellular pathogenesis-related proteins are ribonucleases. Planta, 193: 470-472.

Moiseyev GP, Fedoreyeva LI, Zhuravlev YN, Yasnetskaya E, Jekel PA, Beintema JJ (1997). Primary structures of two ribonucleases from ginseng calluses. New members of the PR-10 family of intracellular pathogenesis-related plant proteins, FEBS Lett. 407: 207-210.

Park CJ, Kim KJ, Shin R, Park JM, Shin YC, Paek KH (2004). Pathogenesis-related protein 10 isolated from hot pepper functions as a ribonuclease in an antiviral pathway. Plant J. 37:186-198.

Poupard P, Parisi L, Campion C, Ziadi S, Simoneau P (2003). A wound- and ethephon-inducible PR-10 gene subclass from apple is differentially expressed during infection with a compatible and an incompatible race of Venturia inaequalis. Physiol. Mol. Plant Pathol. 62: 3-12.

Poupard P, Strull DG, Simoneau P (1998). Two members of the Bet v1 gene family encoding birch pathogenesis-related proteins display different patterns of root expression and wound-inducibility. Aust. J. Plant Physiol. 25: 459-464.

Rakwal R, Agrawal GK, Yonekura M (1999). Separation of proteins from stressed rice *Oryza sativa* L. leaf tissues by twodimensional polyacrylamide gel electrophoresis, induction of pathogenesis-related and cellular protectant proteins by jasmonic acid, UV irradiation and copper chloride. Electrophoresis, 20: 3472-3478.

Robert N, Ferran J, Breda C, Coutos-Thevenot P, Boulay M, Buffard D, Esnault R (2001) Molecular characterization of the incompatible interaction of *Vitis vinifera* leaves with *Pseudomonas syringae* pv. *pisi*: Expression of genes coding for stilbene synthase and class10 PR protein. Eur. J. Plant Pathol.107: 249-261.

Sambrook J, Russell DW (2001). Molecular Cloning: A Laboratory Manual. 3rd ed. New York; Cold Spring Harbor Laboratory Press.

Sels J, Mathys J, De Coninck BMA, Cammue BPA, De Bolle MFC (2008). Plant pathogenesis-related (PR) proteins: A focus on PR peptides. Plant Physiol Biochem 46: 941-950.

Somssich IE, Schmelzer E, Kawalleck P, Halhlbrock K (1988). Gene structure and in situ transcript localization of pathogenesisrelated protein 1 in parsley. Mol. Gen. Genet. 213: 93-98.

Steiner-Lange S, Fischer A, Boettcher A, Rouhara I, Liedgens H, Schmelzer E, Knogge W (2003). Differential defense reactions in leaf tissues of barley in response to infection by Rhynchosporium secalis and to treatment with a fungal avirulence gene product. Mol. Plant Microbe Interact. 16: 893-902.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Van Loon LC, Pierpoint WS, Boller T, Conejero V (1994). Recommen-

dations for naming plant pathogenesis-related proteins. Plant Mol. Biol. Rep. 12: 245-264.
Van Loon LC, Van Strien EA (1999). The families of pathogenesisrelated proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol. 55: 85-97.
Walter MH, Liu JW, Grand C, Lamb CJ, Hess D (1990). Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. Mol. Gen. Genet. 222: 353-360.
Walter MH, Liu JW, Wu¨nn J, Hess D (1996). Bean ribonulease-like pathogenesis-related protein genes Ypr10 display complex patterns of developmental, dark-induced and exogenous-stimulus- dependent expression. Eur. J. Biochem. 239: 281-293.
Wang CS, Huang JC, Hu JH (1999). Characterization of two subclasses of PR-10 transcripts in lily anthers and induction of their genes through separate signal transduction pathways. Plant Mol. Bio. 40: 807-814.
Warner SAJ, Scott R, Draper J (1992). Characterization of woundinduced transcript from the monocot asparagus that share similarity with a class of intracellular pathogenesis-related (PR) proteins. Plant Mol. Biol. 19: 555-561.
Wasternack C (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann. Bot. (Lond) 100: 681-697.
Xu P, Blancaflor EB, Roossinck MJ. (2003). In spite of induced multiple defense responses, tomato plants infected with *cucumber mosaic virus* and D satellite RNA succumb to systemic necrosis. Mol. Plant Microbe Interact 16: 467-476.
Xu XY, Bewley JD, Greenwood JS (2000). Cloning and characterization of an 18 kiloDalton protein in the roots of the perennial weed Taraxacum officinale Weber (Dandelion) which has allergen- and pathogenesis-related protein properties. Plant Cell Environ. 23: 1227-1236.
Zhang R, Wang YC, Liu GF, Li HY (2010) Cloning and characterization of a pathogenesis-related gene (ThPR10) from Tamarix Hispida. Acta Biol. Cracov. Bot. 52: 17-25.
Zhou XJ, Lu S, Xu YH, Wang JW, Chen XY (2002). A cotton cDNA (GaPR-10) encoding a pathogenesis-related 10 protein with *in vitro* ribonuclease activity. Plant Sci. 162: 629-636.