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A Pathogen-Induced Chitin-Binding Protein Gene from Pepper: Its Isolation and Differential Expression in Pepper Tissues Treated with Pathogens, Ethephon, Methyl Jasmonate or Wounding

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A chitin-binding protein (CBP) cDNA (*CACBP1*) was isolated from a cDNA library of pepper (*Capsicum annuum* L.) leaves infected with *Xanthomonas campestris* pv. *vesicatoria*. The deduced amino acid sequence of the *CACBP1* gene which has chitin-binding domain and hinge region shares a high level of identity with CBP sequences from tomato, potato and tobacco. The *CACBP1* gene was organ-specifically regulated in pepper plants, and differentially induced during the compatible and incompatible interactions of pepper with *X. campestris* pv. *vesicatoria* or *Phytophthora capsici*. Expression of the *CACBP1* gene was rapidly induced in the incompatible interactions upon pathogen infection. Transcripts of the *CACBP1* gene was highly inducible in the leaves of matured pepper plants by *Colletotrichum coccodes* infection. In situ hybridization results showed that *CACBP1* mRNA was expressed in the phloem area of vascular bundles in *C. coccodes*-infected leaf tissues. The pathogen-inducible *CACBP1* gene was also strongly induced and accumulated in pepper leaves by ethephon, methyl jasmonate or wounding. These data suggest that ethylene and jasmonate may act as signal molecules in the signal transduction pathways of the CBP gene induction during the pepper defense- or pathogenesis-related plant responses.

Key words: Colletotrichum coccodes — In situ hybridization — Pepper — *Phytophthora capsici* — *Xanthomonas campestris* pv. *vesicatoria*.

Abbreviations: BABA, DL-β-amino-*n*-butyric acid; BTH, benzothiadiazole; HR, hypersensitive response; JA, jasmonic acid; PR, pathogenesis related; SA, salicylic acid; SAR, systemic acquired resistance.

Nucleotide sequence data have been deposited in EMBL/GenBank database under accession number AF112867.

Introduction

As a result of host–pathogen co-evolution, plants have developed elaborate mechanisms to protect themselves from diseases. Besides preformed physical and chemical barriers that hinder infection, a wide variety of defense responses are induced only after pathogen attack (Ramussen et al. 1991, Boch et al. 1998). When these induced responses are triggered rapidly and coordinately during a given plant–pathogen interaction, the plant becomes resistant to disease. A susceptible plant responds more slowly to pathogen infection with an onset of defense mechanisms. Thus, the timely recognition of an invading microorganism, and the rapid and effective induction of defense responses appear to make a key difference between resistant and susceptible plants (Yang et al. 1997).

When pathogens infect plants, the plant may respond by forming a necrotic lesion surrounding the infection site. The biochemical basis of this hypersensitive response (HR) is unknown, but it functions to limit the systemic spread of the pathogen (Dorey et al. 1998). Along with the HR, other cellular processes occur that may reduce primary and secondary pathogen infections, including the production of pathogenesis-related (PR) proteins (Bol and Linthorst 1990, Ward et al. 1991). Among the PR proteins, of particular interest are those with chitinase or 1,3-β-glucanase gene activities (Hennig et al. 1993, Buchter et al. 1997, Hong et al. 2000, Jung and Hwang 2000b). These enzymes are capable of catalyzing the hydrolysis of chitin and 1,3-β-glucan, two structural components of various fungi. A possible role for these enzymes in defense against fungal infection has been suggested by the observation that a basic chitinase in combination with a basic 1,3-β-glucanase has a strong antifungal effect (Schroder et al. 1992).

Only recently have there been investigations into induced resistance for controlling diseases in plants with defined chemicals. It has been known for a long time that salicylic acid (SA) treatment induces disease resistance and systemic acquired resistance (SAR) gene expression in plants (Ward et al. 1991, Malamy and Klessig 1992). A similar activation of the natural plant defense system has been shown to occur upon exogenous application of ethylene (Kieber 1997, Lund et al. 1998), jasmonate, methyl jasmonate (Andersen et al. 1992, Schweizer et al. 1997), DL-β-amino-*n*-butyric acid (Cohen et al. 1994, Sunwoo et al. 1996, Hwang et al. 1997) and benzothiadiazole (BTH) (Benhamou and Benlanger 1998). In all cases, characterization of the biochemical changes associated with chemical-mediated induced resistance revealed a correlation between the establishment of resistance and the accumulation of defense

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CACBP1     MAK---TTALLLSLVLFI-IAAVANAQQCGRQRGGAVCSGSLCCSQYGWCGSTPEYCSPSQGCQSQC-GGSVPT   70
TOMATO     -----------LALVLCISLTSVTNAQQCGRQRGGRLCGGNLCCSQFGWCGSTPEYCSPSQGCQSQCRGGPTPT   74
POTATO1    MVKL-SCGPILLALVLCISLTSVANAQQCGRQRGGALCGNNLCCSQFGWCGSTPEYCSPSQGCQSQCTG-SGPD   73
POTATO2    MVKLISNSTILLSLFLF-SIAAIANAQQCGRQKGGALCSGNLCCSQFGWCGSTPEFCSPSQGCQSQCTGTGGST   74
TOBACCO1   MGKL-ST--LLFALVLYV-IAAGANAQQCGRQRGGALCSGNLCCSQFGWCGSTPEYCSPSQGCQSQCSGGGGGG   71
TOBACCO2   MGKL-ST--LLLVLILYF-IAAGANAQQCGRQRGGALCSGNLCCSQFGWCGSTPEYCSPSQGCQSQCSGGGGGG   71
Consensus  M-K---t--lLlsLvLfi-iaavaNAQQCGRQrGGa-CSg-LCCSQ-GWCGSTPEYCSTSQGCQSQC-Gg--pt

CACBP1     PTPGGGGASAQNVRATYHLYNPQNVGWDLNAVSAYCSTWDANKPLAWRSKYGLTAFCCPVGPRGRDSCGKCLRV 145
TOMATO     PTPGGG-A--Q-VRATYHLYNPQNVGWDLNAVSAYCSTWDANKPYSWRSKYGWTAFCGPVGPRGRDSCGKCLRV 135
POTATO1    PG-QGG--SAQNVRATYHLYNPQNVGWDLNAVSAYCSTWDANKPYAWRSKYGWTAFCGPVGPRGRDSCGKCLRV 145
POTATO2    PTP-SG--SAQNVRATYHIYNPQNVGWDLNAVSAYCSTWDANKPLSWRKKYGWTAFCGPVGPRGRDSCGKCLRV 146
TOBACCO1   GGGGAGGGGAQNVRATYHIYNPQNVGWDLYAVSAYCSTWDGNKPLAWRRKYGWTAFCGPVGPRGRDSCGKCLRV 146
TOBACCO2   G-GGGGGG-AQNVRATYHLYNPQNVGWDLYAVSAYCSTWDGNKPLAWRRKYGWTAFCGPVGPRGRDSCGKCLRV 144
Consenses  ptpggGGasAQNVRATYHLYNPQNVGWDLYAVSAYCSTWDGNKPlaWRsKYG-TAFC-PVGPRGRDSCGKCLRV

CACBP1     TSANTRTGAQTIVRIVDQCGCGNGGLDLDVNVFRQIDTDGVGNQRGHLIVNYQFVDCGDNLNVSL---VIYG   212
TOMATO     T--NTRTGAQTTVRIVDQCSCGNGGLDLDINVFRQIDTDGVGNQQGHLIVNYQFVNCGDNVNVPLLSVVDRE   203
POTATO1    T--NTRTGAQTTVRIVDQCSCGNGGLDLDINVFQQIDTDGVGNQQGHLIVNYQFVDCGDNVNVPLLSVVDKE   213
POTATO2    T--NTRTGAQTTVRIVDQCSCGNGGLDLDVNVFRQIDTDGVGNHQGHLIVNYQFVDCG-------------   200
TOBACCO1   T--NTGTGAQTTVRIVDQCSCGNGGLDLDVNVFRQLDTDGRGNQRGHLIVNYEFVNCGDNMNV-LLSPVDKE   213
TOBACCO2   T--NTGTGAQTTVRIVDQCSCGNGGLDLDVNVFRQLDTDGRGNQRGHLIVNYEFVNCGDNMNV-LLSPVDKE   211
Consensus  T--NTRTGAQTTVRIVDQCSCGNGGLDLDiNVFRQLDTDGVGNqrGHLIVNYqFVdCGDN-NV-L---V---
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Fig. 1 Comparison of amino acid sequences of pepper *CACBP1* (GenBank accession no. AF112867) with other chitin binding proteins (CBP) from other organisms. The deduced amino acid sequences of *CACBP1* from pepper are aligned with the sequences of CBP from tomato (LEUPR4, GenBank accession no. U89764, Harris et al. 1997), potato1 (Win2, GenBank accession no. P09762, Stanford et al. 1989), potato2 (Win1, GenBank accession no. P09761, Stanford et al. 1989), tobacco1 (cbp20–52, GenBank accession no. S72425, Ponstein et al. 1994) and tobacco2 (cbp20–44, GenBank accession no. S72424, Ponstein et al. 1994). In the consensus sequence, residues identical in all of the compared proteins are denoted by capital letters, residues identical in more than two proteins are denoted by lowercase letters, and residues that are not conserved are represented by dashes (-). The chitin-binding domain are boxed. Disulfide linkages are shown at the top line of chitin-binding domain. The putative N-terminal signal peptide cleavage site is indicated by the black arrow head and the C-terminal domains are indicated by underline. Gaps introduced for alignment of homologous regions are indicated by dashes.

molecules (Cohen et al. 1994).

Ethylene is produced when cells undergo necrosis resulting from pathogen infection (Mauch and Staehelin 1989, Mauch et al. 1992). Exogenous application of ethylene induces the accumulation of defense-related proteins, such as phenylalanine ammonia-lyase (PAL) and vacuolar hydrolases (Ecker and Davis 1987, Mauch et al. 1992). The plant-growth regulator jasmonate has been attributed to a number of regulating functions in plant development (Sembdner and Parthier 1993), affecting the physiological process and ethylene biosynthesis when applied exogenously at low concentration (Sembdner and Parthier 1993). The wound and pathogen responses associated with jasmonate include the accumulation of the leaf thionin (Andersen et al. 1992), and defense-related proteins (Rickauer et al. 1997, Schweizer et al. 1997). It has been postulated that jasmonates might constitute lipid-derived messengers in the signal transduction chain preceding the activation of defense gene expression (Farmer et al. 1998, Watanabe and Sakai 1998).

Plants synthesize a wide array of proteins capable of reversibly binding to affinity matrices composed of chitin, a β-1,4-linked biopolymer of *N*-acetylglucosamine. All chitin-binding proteins for which the amino acid sequence is known to contain a common structural motif of 30–43 amino acids with several cysteines and glycines at conserved positions. This polypeptide motif henceforth is referred to as the chitin-binding domain. The biological function of chitin-binding proteins in plants has yet to be conclusively demonstrated. There is evidence for functions of chitin-binding proteins in plant defense, without excluding additional functions in plant development (Raikhel and Lee 1993). First, there is the lack of chitin in plants and its presence in plant pathogens. Second, the conservation of amino acid sequence with chitin-binding activity within this family of proteins indicates an essential role in plant interactions with chitin-containing microorganisms. Chitin-binding proteins, such as hevein (van Parijs et al. 1991), stinging nettle lectin (Broekaert et al. 1989) and some antimicrobial peptides have been shown to exhibit antifungal activity. However, all these chitin-binding proteins do not have detectable levels of chitinase activities.

In this paper, a chitin-binding protein gene (*CACBP1*) was isolated from a cDNA library of pepper (*Capsicum annuum* L.) leaves infected by *Xanthomonas campestris* pv. *vesicatoria* using differential display techniques. Temporal and spatial accumulation of *CACBP1* mRNA in pepper plants during compatible and incompatible interactions with pathogens were examined by Northern blot analysis and in situ hybridization. To gain more insight into the signal transduction of *CACBP1* gene activation, we also analyzed the functions of ethylene and jasmonic acid as signal molecules in the induction of *CACBP1* gene in pepper plants.

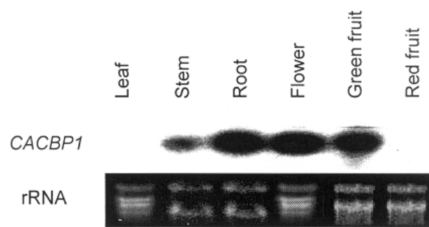

Fig. 2 Northern blot analyses of *CACBP1* mRNA expression in various pepper organs. Total RNA (30 μg) from each sample was loaded in each lane. The *Eco*RI/*Xho*I fragment of putative pepper *CACBP1* cDNA insert in pBluescript SK(–) was labeled and used as a probe. A duplicate gel was stained with ethidium bromide as a control for RNA loading.

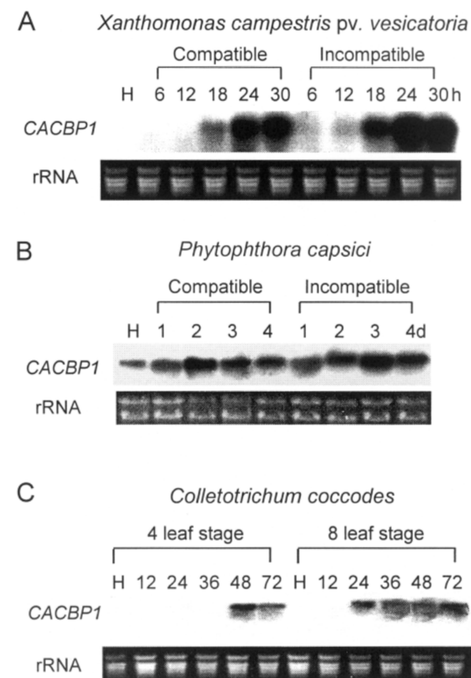

Fig. 3 Time courses of accumulation of transcripts of *CACBP1 cDNA* gene in pepper tissues during the compatible and incompatible interaction of pepper with *Xanthomonas campestris* pv. *vesicatoria*, *Phytophthora capsici* or *Colletotrichum coccodes*. (A) Northern blot analysis of *CACBP1* mRNA in pepper leaves at various times after inoculation with virulent strain Ds1 and avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria.* (B) Northern blot analysis of *CACBP1* mRNA in pepper stems at various times after inoculation with virulent isolate S197 and avirulent isolate CBS178.26 of *P. capsici.* C, Northern blot analysis of *CACBP1* mRNA in pepper leaves at various times after inoculation with strain 2–25 of *C. coccodes* at the 4-leaf and 8-leaf stages. Total RNA (30 μg) from the samples at various time intervals after inoculation was loaded in each lane. The *Eco*RI/*Xho*I fragment of putative pepper *CACBP1* cDNA insert in pBluescript SK(–) was labeled and used as a probe. A duplicate gel was stained with ethidium bromide as a control for RNA loading. D, days after inoculation; H, healthy, mock inoculation; h, h after inoculation.

Results

Isolation and sequence analysis of CACBP1 cDNA

With a partial cDNA clone C19U1 from the differential display PCR as a probe, a new chitin binding protein (CBP) cDNA clone, designated *CACBP1* (*Capsicum annuum* chitin binding protein 1), was isolated from the cDNA library of pepper plants. Sequence analysis of *CACBP1* based on PCGENE program revealed that the ORF encoded a putative protein of 212 amino acids, with an eukaryotic secretion signal sequence (Nielson et al. 1997) locating between amino acid residues 22 and 23 (data not shown). The *CACBP1* cDNA encodes a putative polypeptide of 212 amino acids with a predicted molecular mass of 22,265 Da and a predicted isoelectric point (pI) of 7.99. Analysis of amino acid sequence alignments between the *CACBP1* gene product and GenBank database sequences shows that it is most similar to the proteins encoded by chitin binding protein genes (Altschul et al. 1990) (Fig. 1). The *CACBP1* gene (GenBank accession no. AF112867) product has a high level of homology (80.3–86.5%) with chitin binding proteins from other plants. The *CACBP1* gene product shares 80.3% homology with LEUPR4 from tomato (Harris et al. 1997), 83.1% homology with Win2 from potato (Stanford et al. 1989), 86.5% homology with Win1 (Stanford et al. 1989) from potato, 82.6% homology with cbp20–52 from tobacco (Ponstein et al. 1994) and 84.4% homology with cbp20–44 from tobacco (Ponstein et al. 1994).

Organ-specific localization of CACBP1 mRNA in pepper tissues

RNA gel blot analysis was performed with *CACBP1* cDNA probe to examine whether or not *CACBP1* mRNA was constitutively expressed in leaf, stem, root, flower and fruit tissues of pepper cultivar Hanbyul (Fig. 2). The *CACBP1* mRNAs were not detectable in leaf and red fruit tissues. However, high levels of the transcripts existed in root, flower, and green fruit tissues.

Induction and in situ localization of CACBP1 mRNAs by pathogens

Infection of pepper plants (cultivar Hanbyul) by *X. campestris* pv. *vesicatoria* resulted in a remarkable increase in *CACBP1* mRNA in the infected leaves (Fig. 3A). As shown in Fig. 3A, *CACBP1* mRNAs drastically accumulated during the disease development. In the compatible interactions, the level of *CACBP1* mRNA started to increase 18 h after inoculation. The increase of *CACBP1* transcripts was more pronounced at 24 h after inoculation, thereafter reaching a peak at 30 h. In the incompatible interactions, *CACBP1* mRNAs started to accumulate at 6 to 12 h after inoculation. The accumulation was quite distinct at 18 h after inoculation. *CACBP1* mRNA level peaked at 24 h after inoculation, but decreased at 30 h. In the incompatible interactions, *CACBP1* mRNAs were rapidly and strongly accumulated in the infected leaves, as compared to those in the compatible interactions.

RNA gel blot analysis was performed in pepper stems at various times after inoculation with virulent or avirulent iso-

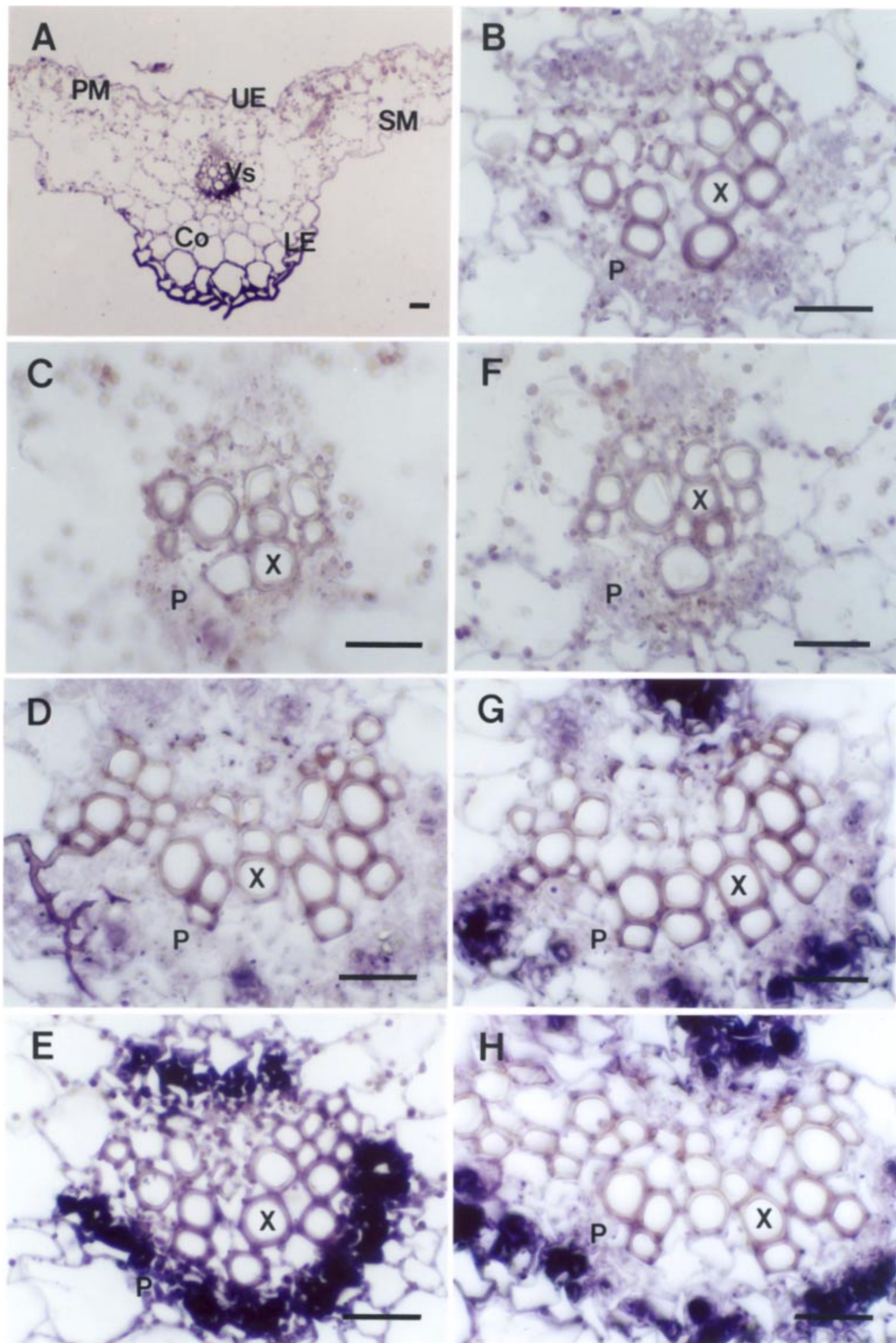

Fig. 4 In situ localization of *CACBP1* mRNAs in midribs of pepper leaves infected by *Colletotrichum coccodes* at 4-leaf and 8-leaf stages. Leaves were sampled from pepper plants at the indicated time intervals. (A) Cross-sections of pepper leaf tissues at 48 h after inoculation. (B) Negative control of in situ hybridization. (C and F) uninoculated leaf tissues, (D and G) 24 h, (E and H) 48 h after inoculation. Thin sections were hybridized with digoxigenin-labeled pepper *CACBP1* cDNA (purple). Co, collenchyma cell; LE, lower epidermis; P, phloem; PM, phallisade mesophyll; SM, sponge mesophyll; UE, upper epidermis; X, xylem; Vs, vascular bundle. Scale bar = 25 μm. (B) to (E), leaf tissues at 4-leaf stage, and (F) to (H), leaf tissues at 8-leaf stage.

late of *P. capsici* (Fig. 3B). The *CACBP1* mRNAs were constitutively expressed in healthy pepper stems, but considerably accumulated after inoculation with *P. capsici*. In the compatible interactions, *CACBP1* mRNAs started to increase 1 d after inoculation. *CACBP1* mRNA level peaked at 2 d after inoculation, and gradually declined thereafter. In the incompatible interactions, the level of *CACBP1* mRNA started to increase 1 d after inoculation. *CACBP1* mRNA level peaked at 3 d after inoculation and decreased at 4 d. In the incompatible interactions, *CACBP1* mRNAs accumulated at a high level, as compared to those in the compatible interactions.

The mRNA expression levels of *CACBP1* were analyzed in the interactions between *C. coccodes* and pepper leaves at 4- and 8-leaf stages (Fig. 3C). *CACBP1* mRNAs were not expressed in the healthy leaves at 4- and 8-leaf stages. In the pepper leaf tissues at 4-leaf stage, the transcripts reach a high level at 48 h after inoculation, and the transcript level declined at 72 h. At the 8-leaf stage, the transcripts began to accumulate at 24 h after inoculation and remained at a high level to 72 h. The specific expression of *CACBP1* mRNA in the infected pepper leaf tissues enabled us to perform a more detailed examination of *CACBP1* mRNA localization in the infected leaf tissues. We examined the spatial and temporal expression patterns of the *CACBP1* mRNA in midribs of the leaves infected by *C. coccodes* using the in situ hybridization technique (Fig. 4). In situ hybridization with a *CACBP1* Dig-labeled cDNA probe showed that *CACBP1* mRNA accumulated massively around infected sites and phloem cells (Fig. 4A). At the 4- and 8-leaf stages, the *CACBP1* mRNAs were not detectable in uninoculated leaf tissues (Fig. 4C, F). At the 4-leaf stage, the *CACBP1* mRNA began to accumulate at 24 h after inoculation (Fig. 4D). At the 8-leaf stage, local mRNA accumulation was clearly visible as early as 24 h after inoculation (Fig. 4G). The transcripts continued to accumulate at 48 h after inoculation (Fig. 4E, H), especially in the compatible interaction. No transcripts, above background levels, were observed when no probe was used (Fig. 4B). The in situ hybridization results were consistent with those of the RNA gel blot analysis (Fig. 3C).

Induction of CACBP1 mRNA by abiotic elicitors

To determine the involvement of abiotic elicitors in *CACBP1* gene induction, pepper plants were treated with abiotic elicitors. The *CACBP1* mRNA expression was monitored in pepper leaves that were incubated for 24 h after treatment with abiotic elicitors. The transcripts was not detectable in the healthy, control pepper leaves. Treatment with ethephon (10 mM) and methyl jasmonate (100 μM) greatly induced accumulation of *CACBP1* mRNAs (Fig. 5A). However, DL-β-amino-*n*-butyric acid (19.4 mM) and benzothiadiazole (95.2 μM) slightly induced *CACBP1* transcripts, whereas salicylic acid (5 mM) did not induce transcripts at all.

The effects of time of ethephon treatment were further examined for the induction of the *CACBP1* mRNA (Fig. 5B). To analyze the response of pepper plants to ethylene, the plants were incubated in the presence of 10 mM ethephon. Figure 5B shows that the *CACBP1* mRNA was induced by treatment with ethephon. Ethephon induced accumulation of *CACBP1* mRNA in a time-dependent manner. The transcripts were not detectable in the healthy, control pepper leaves and in the ethephon-

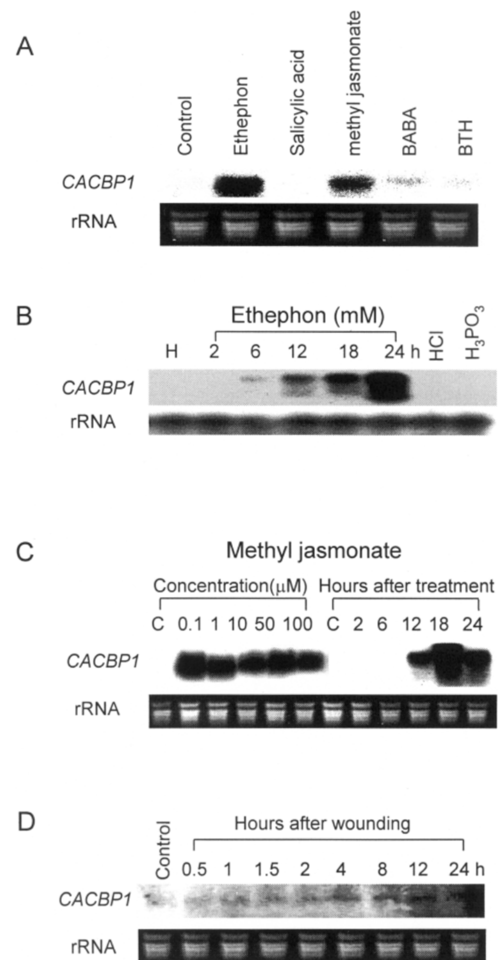

Fig. 5 Northern blot analyses of *CACBP1* mRNA expression in the pepper leaves in response to various abiotic elicitors. Total RNA (30 μg) from each sample was loaded in each lane. The *Eco*RI/*Xho*I fragment of putative pepper *CACBP1* cDNA insert in pBluescript SK(–) was labeled and used as a probe. A duplicate gel was stained with ethidium bromide as a control for RNA loading. (A) *CACBP1* mRNA expression in leaf tissues at 24 h after treatment with each of ethephon (10 mM), salicylic acid (5 mM), methyl jasmonate (100 μM), DL-β-amino-*n*-butyric acid (19.4 mM) and benzothiadiazole CGA245704 (95.2 μM). (B) *CACBP1* mRNA expression in leaf tissues at different time intervals after treatment with ethephon, hydrochloric acid or phosphoric acid. C, control; h, h after treatment. (C) *CACBP1* mRNA expression in leaf tissues at 12 h after treatment with methyl jasmonate at various concentrations, and at various times after treatment with 100 μM methyl jasmonate. C, control. (D) *CACBP1* mRNA expression in leaf tissues at different time intervals after wounding.

treated leaves at 2 h after treatment. The *CACBP1* mRNA began to be slightly induced in pepper leaves 6 h after treatment. The accumulation became more distinct by 18 h after treatment. *CACBP1* mRNA level reached a peak at 24 h after treatment (Fig. 5B). Hydrochloric acid (HCl) and phosphonic acid (H_3PO_3) as breakdown products of ethephon when applied to pepper plants did not induce transcripts of *CACBP1*, indicating that the accumulation of its transcripts was only induced by gaseous ethylene released from the ethephon treated plants.

Induction patterns of *CACBP1* transcripts were examined in pepper leaves at 12 h after treatment with methyl jasmonate of various concentrations and at various times after treatment with 100 μM methyl jasmonate (Fig. 5C). Methyl jasmonate induced accumulation of *CACBP1* mRNA in a dose-independent and time-dependent manner (Fig. 5C). The transcripts were not detectable in the healthy, control leaves and in the methyl jasmonate-treated leaves at 2 and 6 h after treatment. The *CACBP1* mRNA accumulated to a highly constant level upon treatment with methyl jasmonate, irrespective of the concentrations treated. The transcripts began to be induced in pepper leaves at 12 h after treatment, peaked at 18 h, and declined at 24 h after treatment.

Mechanical injury has been shown to trigger the activation of a large array of genes (Bowles 1991, Titarenko et al. 1997). To ascertain the time course of induction of *CACBP1* mRNA upon wounding, Northern analysis was performed with total RNA from both control and wounded leaves. As shown in Fig. 5D, *CACBP1* mRNAs were induced in pepper leaves upon wounding. The *CACBP1* transcripts started to accumulate 1 h after wounding and the accumulation gradually increased by 24 h.

Discussion

The *CACBP1* cDNA was found to be a chitin-binding protein gene that has not been detected so far in pepper plants. The *CACBP1* cDNA codes for the protein containing a signal sequence, chitin-binding domain and C-terminal domain. The chitin-binding domain of *CACBP1* shows extensive identity to those of other proteins like the class I chitinases (Linthorst et al. 1990) and the putative win proteins from potato (Stanford et al. 1989). All chitin-binding proteins for which the amino acid sequence is known contain several cysteines and glycines at conserved positions (Raikhel and Lee 1993). The chitin-binding homologous region in the *CACBP1* polypeptides is preceded by a stretch of 22 amino acids characteristic of a transmembrane leader sequence (von Heijne 1985). This N-terminal domain is highly hydrophobic and its amino acid constituents closely resemble those predicted for signal peptides (von Heijne 1985, von Heijne 1986).

Many of the natural defense mechanisms of plants against microbial infection are induced in response to the signals generated during the initial stages of the host-pathogen interactions (Lamb et al. 1989). In our present study, the pepper leaf tissues infected by avirulent strain Bv5-4a showed HR at 18–24 h after inoculation. In the incompatible interactions, a strong increase in *CACBP1* gene expression occurred at the early stage of infection triggering the HR. The time course of expression of defense-related genes correlates with the paradigm that in an incompatible interaction it is the speed and intensity of the defense reaction that determines resistance to the pathogen (de Wit and van der Meer 1986). Because chitin-binding proteins have antifungal activity, rapid and strong expression of *CACBP1* mRNA in the incompatible interactions may give the pepper plant an advantage over the pathogen during the early stage of infection.

The *CACBP1* transcripts were constitutively expressed in pepper stems. Constitutive overexpression of *CACBP1* gene may contribute to the protection of pepper plants against pathogen attacks. However, the transcripts were also strongly induced in the stem tissues infected with *P. capsici*. In the compatible interactions, the expression of transcripts reached a maximum at 2 d after inoculation, but gradually decreased thereafter. In the incompatible interactions, the transcript level was higher than that in the compatible interactions during the disease development. Therefore, strong induction of *CACBP1* mRNA in the incompatible interaction seemed to contribute to the expression of defense response to *P. capsici*. The symptoms on pepper stems infected by *P. capsici* were different between virulent and avirulent isolates. The virulent isolate that may colonize rapidly into plant tissue caused deterioration of infected tissues. In contrast, the avirulent isolate that may colonize slowly in plant tissue caused incompatible responses with the brownish-purple lesions.

In the interactions between pepper and *C. coccodes*, RNA gel blot and in situ hybridization demonstrated an earlier *CACBP1* mRNA expression in the 8-leaf stage rather than the 4-leaf stage. The early and intense accumulation of the *CACBP1* mRNA in the incompatible interaction between 8-leaf stage pepper and *C. coccodes* may be significant in the expression of plant defense against pathogen. The in situ hybridization experiments with infected leaf tissues demonstrated that the *CACBP1* mRNA accumulation occurs massively within the pathogen penetration sites and phloem cells. Our results were consistent with the data obtained for other PR protein genes, such as PR-1, chitinase, PR-10, win (wound induced gene) and thionin, which exhibited strong expression in vascular bundles (Stanford et al. 1989, Zhu et al. 1993, Breda et al. 1996, Lee et al. 2000a, Lee et al. 2000b, Lee et al. 2000c) and penetration sites (Somssich et al. 1998).

Some abiotic elicitors such salicylic acid, ethylene, jasmonic acid (JA) and BTH have been known to be important for the induction of disease resistance through signaling pathways in plants. The effects of SA, jasmonate and BTH have been studied following the application to cucumber, tobacco and Arabidopsis (Metraux et al. 1990, Lawton et al. 1996, Rickauer et al. 1997). Northern blot analysis was performed with *CACBP1* cDNA probe to examine the expression patterns

in pepper leaves treated with abiotic elicitors such as ethephon, salicylic acid, methyl jasmonate, BABA and BTH. Treatment of pepper plants with ethylene-releasing ethephon and methyl jasmonate strongly induced the expression of *CACBP1* mRNA. These results were well consistent with the previous findings that the PR proteins induced by ethylene also were induced by methyl jasmonate (Xu et al. 1994, Buchter et al. 1997). More recently, we have demonstrated that infection by the avirulent strain of *X. campestris* pv. *vesicatoria* triggered concomitantly PR-1 mRNA expression and the ethylene biosynthesis in pepper leaves, indicating that the basic PR-1 mRNA induction pattern was very similar to ethylene biosynthesis pattern (Kim and Hwang 2000). Ethephon treatment also induced strongly the expression of the basic PR genes in pepper leaves (Kim and Hwang 2000, Jung and Hwang 2000a). It seems likely that the rates of ethylene biosynthesis by either pathogen infection or ethephon treatment are directly proportional to those of the basic PR protein accumulation. Exogenous application of methyl jasmonate induced the accumulation of PR protein transcripts in pepper (Jung and Hwang 2000a), which suggests a role for methyl jasmonate in mediating defense-related gene induction in pepper plants. A dose-response study of *CACBP1* mRNA induction by methyl jasmonate revealed that relatively low concentrations of methyl jasmonate were required for the maximum expression of *CACBP1* mRNA. Methyl jasmonate treatment induced accumulation of *CACBP1* mRNA in pepper leaves, independently of treatment concentrations. These data strongly suggested that an elevated jasmonate concentration may not be the extracellular signal in response to pathogen infection that activates defense reactions. These observations are well supported by the recent suggestions of Steiner and Schönbeck (1997) that abiotic elicitors induced PR proteins in plants, regardless of the treated concentration. However, the expression pattern of *CACBP1* in pepper leaves was time-dependent after treatment with methyl jasmonate. The involvement of JA in regulating gene activation upon wounding has been firmly established in several plant species (Creelman et al. 1992, Hildmann et al. 1992, Laudert et al. 1996). The wound signaling has two different pathways; via a JA-dependent or independent pathway (Titarenko et al. 1997). Expression of some CBP genes that have a high level of homology with *CACBP1* in pepper were found to be wound-inducible in potato (Stanford et al. 1989), tobacco (Ponstein et al. 1994) and tomato (Harris et al. 1997). As shown in Fig. 5D, the *CACBP1* transcripts started to accumulate 1 h after wounding. These results suggest that *CACBP1* gene expression may occur in JA-dependent pathway and the wound response in the pepper plants may involve the activation of *CACBP1* gene expression.

Materials and Methods

Plant materials

Seeds of pepper (*Capsicum annuum* L. cv. Hanbyul) were sown in a plastic tray (55×35×15 cm) containing steam-sterilized soil mix of a commercial compost soil (peat moss, perlite, and vermiculite, 5 : 3 : 2, v/v/v), sand and loam soil (1 : 1 : 1, v/v/v). Six pepper seedlings at the two-leaf stage were transplanted to each of plastic pots (5×15×10 cm) containing the above described soil mix. Pepper plants were raised in a growth room at 27±1°C with approximately 80 µmol m^{-2} s^{-1} (white fluorescent lamps) for 16 h a day.

Pathogen inoculation

The two strains Ds1 and Bv5-4a of *Xanthomonas campestris*. pv. *vesicatoria*, which were virulent and avirulent to the pepper cultivar Hanbyul, respectively, were used in this study (Kim and Hwang 2000). To prepare bacterial suspensions for inoculation on pepper leaves, bacterial strains were cultured overnight on the yeast-nutrient (YN) broth (5 g yeast extract, 8 g nutrient broth and 1 liter H_2O) at 28°C. Cell suspensions were adjusted to 10^8 colony forming units (CFU) ml^{-1} with sterile tap water prior to inoculation (Lee et al. 2000a). Pepper plants at 6-leaf stage were inoculated by vacuum-infiltrating bacterial suspensions into the abaxial side of fully expanded leaves with an atomizer. The control healthy plants also were mock-inoculated by infiltrated sterile tap water into the leaves. The mock-inoculated and inoculated pepper plants were incubated in a growth room as described previously (Lee and Hwang 1996).

Two isolates S197 and CBS 178.26 of *Phytophthora capsici* which were evaluated virulent and avirulent to pepper cultivar Hanbyul, respectively (Kim and Hwang 1994), were grown on oatmeal agar plates for 10 d and then induced to sporulate under fluorescent light for 2 d at 28°C. Zoospores released from the sporangia of *P. capsici* were adjusted to 10^5 zoospores ml^{-1} with sterile tap water. A small quantity of cotton soaked in zoospore suspensions was placed on the bottom region of each pepper stem. The inoculated pepper plants were incubated in a growth room as described previously (Kim and Hwang 1994).

The isolate 2–25 of *Colletotrichum coccodes* obtained from Plant Pathology Division, National Agricultural Sciences & Technology Institute in Korea was used in this study. The fungus was grown on oatmeal agar plates for 5–7 d at 28°C under fluorescent light. Fungal inocula were prepared for inoculation on the pepper leaves, as previously described by Hong and Hwang (1998). Conidia concentration was adjusted to 10^6 ml^{-1} with sterile tap water using a hemacytometer. Before inoculation, Tween 20 was added to the inoculum to give 0.05% (v/v) final concentration. Pepper plants at the 4- and 8-leaf stages were inoculated with the conidial suspension using a foliar spray method. The inoculated pepper plants were incubated in a moist chamber for 36 h in the dark at 28°C and then returned to the growth room.

Abiotic elicitor and wounding treatment

Chemicals were applied as a spray to pepper plants at 6-leaf stage with a solution of 0.01% Tween 20 containing each of the following: 10 mM ethephon, 5 mM salicylic acid, 0.1, 1, 10, 50, 100 µM methyl jasmonate, 19.4 µM DL-β-amino-*n*-butyric acid and 95.2 µM benzothiadiazole CGA245704. Pepper plants applied with either of ethephon and methyl jasmonate were incubated in a vinyl bag. Control plants were sprayed with water. The treated pepper plants were incubated in a growth room as described previously (Kim and Hwang 1994).

Leaves of pepper plants at 6-leaf stage were scraped using sterile needles for wounding. The wounded pepper plants were incubated in a growth room at 27±1°C with approximately 80 µmol m^{-2} s^{-1} (white fluorescent lamps) for 16 h a day.

Differential display

To search for differentially expressed cDNAs, the RNA extracted

from the healthy or *X. campestris* pv. *vesicatoria*-infected pepper leaf tissues was used for reverse transcription in a 20 µl reaction volume. The cycling parameters for PCR reactions were as follows: 94°C for 30 s, 40°C for 2 min, 72°C for 30 s for 40 cycles followed by 72°C for 5 min. Reaction products were separated on 6% denaturing polyacrylamide gel, dried and visualized by X-ray film. After developing the X-ray films, the bands of cDNA genes that code for mRNAs induced only in the leaves infected by *X. campestris* pv. *vesicatoria* were excised from the polyacrylamide gel and amplified. One of the cDNAs, designated C19U1, was cloned into TA cloning system from Invitrogen (San Diego, CA, U.S.A.) to use as a probe for screening the cDNA library of pepper.

Construction and screening of a cDNA library

For the construction of a pathogen-induced cDNA library, total RNA was isolated from pepper plants infected by the incompatible strain Bv5-4a of *X. campestris* pv. v*esicatoria*. Poly(A)$^+$-RNA was isolated from the total RNA by oligo(dT)-cellulose chromatography (Sambrook et al. 1989). The cDNA library was constructed from 5 µg mRNA using a λZAPII-cDNA synthesis kit (Stratagene). The cDNA library, which had a titer of approximately 10^8 plaque-forming units (PFU) μg^{-1} insert DNA, was transferred to nylon membranes and probed with the C19U1 cDNA. Some clones with ca. 0.7 kb inserts were isolated from the cDNA library.

DNA sequencing

C19U1 clone was sequenced on an automated DNA sequencer (model ABI 310 DNA sequencer; Applied Biosystem). The sequence of *CACBP1* cDNA was determined from deletion derivates of the plasmid generated by exonuclease III and S1 nuclease digestion. The sequence analysis was carried out using the PC/Gene software system and BLAST network services at the National Center for Biotechnology Information (Altschul et al. 1990). The amino acid alignments were manually adjusted to compare *CACBP1* cDNA clones with those of other organisms.

RNA isolation and gel blot analysis

Total RNA was prepared from pepper leaves, stems, roots, flowers and fruits by the guanidine isothiocyanate method (Chomczynski and Sacchi 1987). The RNA was stored in DEPC-treated sterile water at −70°C until used. Thirty micrograms of RNA were denatured and separated on 1.2% agarose-formaldehyde gels, transferred onto nylon membranes (Hybond N$^+$, Amersham) according to the conditions recommended by the manufactures. The blotted RNA was cross-linked to the membrane by UV illumination. ^{32}P-labeled *CACBP1* probe was made by a random prime kit (Boehringer Mannheim). Blots were prehybridized for 2 h at 65°C in 5% (w/v) dextran sulfate, 0.25 M disodium phosphate pH 7.2, 7% (w/v) sodium dodecyl sulfate (SDS), and 1 mM EDTA. Hybridization was performed for 16 h at 65°C using ^{32}P-labeled probes made with the random prime kit (Boehringer Mannheim) and cytosine 5′ [α-^{32}P]triphosphate (Amersham). The membranes were washed twice with 2× SSC and 0.1% SDS for 10 min each at room temperature, several times with 0.1× SSC and then 0.1% SDS for 5 min each at 65°C.

In situ RNA localization

In situ localization was conducted as described by Cox and Goldberg (1988) and van de Wiel et al. (1990). Plant samples (5×5 mm) from pepper leaves inoculated with *C. coccodes* at the 4- and 8-leaf stages were fixed in 30 mM sodium phosphate and 130 mM sodium chloride (1× PBS) buffer, containing 4% paraformaldehyde and 1 µl ml^{-1} Triton X-100 by vacuum infiltration and shaking for 2 h. Fixed samples were washed with 1× PBS buffer, dehydrated in a graded ethanol and xylene series, and embedded in paraplast.

Tissue sections (10 µm in thickness) were cut from the diseased sites and attached to poly-L-lysine-coated microscopic slides. After removal of the paraplast with xylene and rehydration through an ethanol series, the sections were treated with 1% bovine serum albumin (BSA) in 10 mM Tris-Cl pH 8.0, proteinase K (5 mg ml^{-1}) in 50 mM EDTA, 100 mM Tris-HCl pH 8.0 for 30 min at 37°C and 0.25% acetic anhydride in 100 mM triethanolamine pH 8.0 for 10 min at room temperature. The *Eco*RI/*Xho*I insert carrying the *CACBP1* cDNA was digoxigenin-labeled with a Dig High prime kit (Boehringer Mannheim). Sections were prehybridized and hybridized with digoxigenin-labeled probes in 50% formamide, containing 4× SSC, 0.5% blocking reagent, 150 µg ml^{-1} tRNA for 18 h at 42°C. After hybridization, the sections were rinsed twice with 50% formamide and 4× SSC, twice 4× SSC and then DEPC-sterile water at 42°C. The hybridization signals were detected with the diluted nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate at room temperature following the procedures described by suppliers (Boehringer Mannheim).

To demonstrate the specificity of the hybridization, the control tests were performed. The level of non-specific hybridization of the *CACBP1* probe was evaluated by omitting the *CACBP1* probe from the standard protocol. The specificity of the *CACBP1* probe also was examined on an uninfected, healthy or infected plant specimen.

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References

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

Andersen, I., Becker, W., Schluter, K., Burges, J., Parthier, B. and Apel, K. (1992) The identification of leaf thionin as one of the main jasmonate-induced proteins of barley (*Hordeum vulgare*). *Plant Mol. Biol.* 19: 193–204.

Benhamou, N. and Benlanger, R.R. (1998) Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato. *Plant Physiol.* 118: 1203–1212.

Boch, J., Verbsky, M.L., Robertson, T., Larkin, J.C. and Kunkel, B.N. (1998) Analysis of resistance gene-mediated defense response in *Arabidopsis thaliana* plants carrying a mutation in *CPR5*. *Mol. Plant Microbe Interact.* 12: 1196–1206.

Bol, J.F. and Linthorst, H.J.M. (1990) Plant pathogenesis-related proteins induced by virus infection. *Annu. Rev. Phytopathol.* 28: 113–138.

Breda, C., Sallaud, C., El-Turk, J., Buffare, D., de Kozak, I., Esnault, R. and Kondorosi, A. (1996) Defense fraction in *Medicago sativa*: a gene encoding a class to PR protein is expressed in vascular bundles. *Mol. Plant Microbe Interact.* 8: 713–719.

Broekaert, W.F., Van Parijs, J., Leyns, F., Joos, H. and Peumans, W.J. (1989) A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science* 245: 1100–1102.

Buchter, R., Stromberg, A., Schmelzer, E. and Kombrink, E. (1997) Primary structure and expression of acidic (class II) chitinase in potato. *Plant Mol. Biol.* 35: 749–761.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156–159.

Cohen, Y., Niderman, T., Mosinger, E. and Fluhr, R. (1994) β-Aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (*Lycopersicon esculentum* L.) plants and resistance to late blight infection caused by *Phytophthora infestans*. *Plant Physiol.* 104: 59–66.

Cox, K.H. and Goldberg, R.B. (1988) Analysis of plant gene expression. *In* Plant Molecular Biology: A Practical Approach. Edited by Shaw, C.H. pp. 1–34. IRL Press, Oxford.

Creelman, R.A., Tierney, M.L. and Muller, J.E. (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. *Proc. Natl. Acad. Sci. USA* 89: 4938–4941.

de Wit, P.J.G.M. and van der Meer, F.E. (1986) Accumulation of the pathogenesis-related tomato leaf protein P14 as an early indicator of incompatibility in the interaction between *Cladosporium fulvum* (Syn. *Fulvia fulva*) and tomato. *Physiol. Mol. Plant Pathol.* 28: 203–214.

Dorey, S., Baillieul, F., Saindrenan, P., Fritig, B. and Kauffmann, S. (1998) Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. *Mol. Plant Microbe Interact.* 11: 1102–1109.

Ecker, J.R. and Davis, R.W. (1987) Plant defense genes are regulated by ethylene. *Proc. Natl. Acad. Sci. USA* 84: 5202–5206.

Farmer, E.E., Weber, H. and Vollenweider, S. (1998) Fatty acid signaling in Arabidopsis. *Planta* 206: 167–174.

Harris, N., Taylor, J.E. and Roberts, J.A. (1997) Characterization and expression of a mRNA encoding a wound-induced (win) protein from ethylene-treated tomato leaf abscission zone tissue. *J. Exp. Bot.* 48: 1223–1227.

Hennig, J., Dewey, R.E., Cutt, J.R. and Klessig, D.F. (1993) Pathogen, salicylic acid and developmental dependent expression of a β-1,3-glucanase/GUS gene fusion in transgenic tobacco plants. *Plant J.* 4: 481–493.

Hildmann, T., Ebneth, M., Peña-Cortés, H., Sánchez-Serrano, J.J., Willmitzer, L. and Prat, S. (1992) General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. *Plant Cell* 4: 1157–1170.

Hong, J.K. and Hwang, B.K. (1998) Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum coccodes*. *Plant Dis.* 82: 1079–1083.

Hong, J.K., Jung, H.W., Kim, Y.J. and Hwang, B.K. (2000) Pepper gene encoding a basic class II chitinase is inducible by pathogen and ethephon. *Plant Sci.* 159: 39–49.

Hwang, B.K., Sunwoo, J.Y., Kim, Y.J. and Kim, B.S. (1997) Accumulation of β-1,3-glucanase and chitinase isoforms, and salicylic acid in the DL-β-amino-*n*-butyric acid-induced resistance response of pepper stems to *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 51: 305–322.

Jung, H.W. and Hwang, B.K. (2000a) Isolation, partial sequencing, and expression of pathogenesis-related cDNA genes from pepper leaves infected by *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Plant Microbe Interact.* 13: 136–142.

Jung, H.W. and Hwang, B.K. (2000b) Pepper gene encoding a basic β-1,3-glucanase is differentially expressed in pepper tissues upon pathogen infection and ethephon or methyl jasmonate treatment. *Plant Sci.* 159: 97–106.

Kieber, J.J. (1997) The ethylene response pathway on Arabidopsis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 277–296.

Kim, Y.J. and Hwang, B.K. (1994) Differential accumulation of β-1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 45: 185–209.

Kim, Y.J. and Hwang, B.K. (2000) Pepper gene encoding a basic pathogenesis related 1 protein is pathogen and ethylene inducible. *Physiol. Plant.* 108: 51–60.

Lamb, C.J., Lawton, M.A. and Dixon, R.A. (1989) Signal and transduction mechanisms for activation of plant defenses against microbial attack. *Cell* 56: 215–224.

Laudert, D., Pfannschmidt, U., Lottspeich, F., Hollander-Czytko, H. and Weiler, E.W. (1996) Cloning, molecular and functional characterization of *Arabidopsis thalinana* allene oxide synthase (CYP74), the first enzyme of the octacanoid pathway to jasmonates. *Plant Mol. Biol.* 31: 323–335.

Lawton, K., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann. H., Staub, T. and Ryals, J. (1996) Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* 10: 71–82.

Lee, S.C., Hong, J.K., Kim, Y.J. and Hwang, B.K. (2000a) Pepper gene encoding thionin is differentially induced by pathogens, ethylene and methyl jasmonate. *Physiol. Mol. Plant Pathol.* 56: 207–216.

Lee, S.C., Lee, Y.K., Kim, K.D. and Hwang, B.K. (2000b) In situ hybridization study of organ- and pathogen-dependent expression of a novel thionin gene in pepper (*Capsicum annuum*). *Physiol. Plant.* 110: 384–392.

Lee, Y.K., Hippe-Sanwald, S., Lee, S.C., Hohenberg, H. and Hwang, B.K. (2000c) In situ localization of PR-1 mRNA and PR-1 protein in compatible and incompatible interactions of pepper stems with *Phytophthora capsici*. *Protoplasma* 211: 64–75.

Lee, Y.K. and Hwang, B.K. (1996) Differential induction and accumulation of β-1,3-glucanase and chitinase isoforms in the intercellular space and leaf tissues of pepper by *Xanthomonas campestris* pv. *vesicatoria* infection. *J. Phytopathol.* 144: 79–87.

Linthorst, H.J.M., Van Loon, L.C., Van Rossum, C.M.A., Mayer, A., Bol, J.F., Van Roekel, J.S.C., Meulenhoff, E.J.S. and Cornelissen, B.J.C. (1990) Analysis of acidic and basic chitinases from tobacco and petunia and their constitutive expression in transgenic tobacco. *Mol. Plant Microbe Interact.* 3: 252–258.

Lund, S.T., Stall, R.E. and Klee, H.J. (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. *Plant Cell* 10: 371–382.

Malamy, J. and Klessig, D.F. (1992) Salicylic acid and plant disease resistance. *Plant J.* 2: 643–654.

Mauch, F., Meehl, J.B. and Staehelin, L.A. (1992) Ethylene-induced chitinase and β-1,3-glucanase accumulate specifically in the lower epidermis and along vascular strands of bean leaves. *Planta* 186: 367–375.

Mauch, F. and Staehelin, L.A. (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and β-1,3-glucanase in bean leaves. *Plant Cell* 1: 447–457.

Metraux, J.P., Signar, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschcorf, K., Schmid, E., Blum, W. and Inverardi, B. (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250: 1004–1006.

Ponstein, A.S., Bres-Vloemans, S.A., Sela-Buurlage, M.B., van den Elzen, P.J.M., Melchers, L.S. and Cornelissen, B.J.C. (1994) A novel pathogen- and wound-inducible tobacco (*Nicotiana tabacum*) protein with antifungal activity. *Plant Physiol.* 104: 109–118.

Raikhel, N.V. and Lee, H.I. (1993) Structure and function of chitin-binding proteins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44: 591–615.

Ramussen, J.B., Hammerschmidt, R. and Zook, M.N. (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*. *Plant Physiol.* 97: 1342–1347.

Rickauer, M., Brodschelm, W., Bottin, A., Veronesi, C., Grimal, H. and Esquerre-Tugaye, M.T. (1997) The jasmonate pathway is involved differentially in the regulation of different defence responses in tobacco cells. *Planta* 202: 155–162.

Sambrook, J., Fritsch, E.F. and Maniatis, R. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schroder, M., Hahlbrock, K. and Kombrink, E. (1992) Temporal and spatial patterns of 1,3-β-glucanase and chitinase induction in potato leaves infected by *Phytophthora infestans*. *Plant J.* 2: 161–172.

Schweizer, P., Buchala, A. and Metraux, J.P. (1997) Gene-expression patterns and levels of jasmonic acid in rice treated with the resistance inducer 2,6-dichloroisonicotinic acid. *Plant Physiol.* 115: 61–70.

Sembdner, G. and Parthier, B. (1993) The biochemistry and the physiological and molecular actions of jasmonates. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44: 569–589.

Somssich, I.E., Schmelzer, E., Kawalleck, P. and Hahlbrock, K. (1998) Gene structure and in situ transcript localization of pathogenesis-related protein 1 in parsley. *Mol. Gen. Genet.* 213: 93–98.

Stanford, A., Bevan, M. and Northcote, D. (1989) Differential expression within a family of novel wound-induced genes in potato. *Mol. Gen. Genet.* 215: 200–208.

Steiner, U. and Schönbeck, F. (1997) Induced resistance. *In* Resistance of Crop Plants Against Fungi. Edited by Hartleb, H., Heitefuss, R. and Hoppe, H.H. pp. 272–297. Gustav Fischer, Jena, Stuttgart, Lubeck, Ulm.

Sunwoo, J.Y., Lee, Y.K. and Hwang, B.K. (1996) Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-*n*-butyric acid. *Eur. J. Plant Pathol.* 102: 663–670.

Titarenko, E., Rojo, E., Leó, J. and Sánchez-Serrano, J.J. (1997) Jasmonic acid-dependent and -independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. *Plant Physiol.* 115: 817–826.

van de Wiel, C., Scheres, B., Franssen, H., Van Lierop, M.J., Van Lammeren, A., Van Kammen, A. and Bisseling, T. (1990) The early nodulin transcript ENDO2 is located in the module parenchyma (inner cortex) of pea soybean root nodules. *EMBO J.* 9: 1–7.

van Parijs, J., Broekaert, W.F., Goldstein, I.J. and Peumans, W.J. (1991) Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. *Planta*

183: 258–264.

von Heijne, G. (1985) Signal sequences: the limits of variation. *J. Mol. Biol.* 184: 99–105.

von Heijne, G. (1986) A new method for predicting signal sequence cleavage sites. *Nucl. Acids Res.* 14: 4683–4690.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Metraux, J. and Ryals, J.A. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3: 1085–1094.

Watanabe, T. and Sakai, S. (1998) Effects of active oxygen species and methyl jasmonate on expression of the gene for a wound-inducible 1-aminocyclopropane-1-carboxylate synthase in winter squash (*Cucurbita maxima*). *Planta* 206: 570–576.

Xu, Y., Chang, P.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M. and Bressan, R.A. (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* 6: 1077–1085.

Yang, Y., Shah, J. and Klessig, D.F. (1997) Signal perception and transduction in plant defense responses. *Genes Dev.* 11: 1621–1639.

Zhu, Q., Doerner, P.W. and Lamb, C.J. (1993) Stress induction and developmental regulation of a rice chitinase promoter in transgenic tobacco. *Plant J.* 3: 203–212.

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