Pepper gene encoding a basic class II chitinase is inducible by pathogen and ethephon

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Abstract

A chitinase cDNA clone (designated CACHI2) was isolated from the cDNA library of pepper leaves infected with Xanthomonas campestris pv. vesicatoria. The 1004-bp full-length CACHI2 cDNA encodes a basic chitinase with an N-terminal 24 amino acid signal peptide followed by a catalytic region. An analysis of its sequence indicates that CACHI2 is a class II chitinase, because it does not have chitin-binding domain and C-terminal extension sequences. The deduced amino acid sequence of CACHI2 has a high level of identity with class II chitinases from potato, tomato, tobacco and petunia. Southern analysis demonstrated that the CACHI2 chitinase is encoded by a single or two copy genes in the pepper genome. Following X. campestris pv. vesicatoria or Phytophthora capsici infection, the CACHI2 chitinase mRNA was more highly expressed in the incompatible interaction, compared to expression in the compatible interaction. Treatment with ethylene-releasing ethephon resulted in a strong accumulation of the transcripts in the leaves. In contrast, DL-β-amino-n-butyric acid, salicylic acid and methyl jasmonate were not effective in inducing CACHI2 transcripts in pepper leaves. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Capsicum annuum; Chitinase cDNA; mRNA expression; Pathogen; Abiotic elicitors

1. Introduction

Chitinase is one of the most important enzymes acting as components of pathogenesis-related mechanisms in plants. Since its natural substrate, chitin (a β-1,4-linked biopolymer of N-acetylglucosamine), is a major structural component of fungal cell walls [1], the induction in plants of chitinases with antifungal activity in vitro may also act as defenses against fungal pathogens [2,3]. The level of chitinase activity is dramatically induced by wounding or infection of plants with fungal, bacterial, or viral pathogens [4–6]. Chitinase activity is also induced in plants by exogenously applied chemicals such as mercuric chloride [3,7]. Purified plant chitinases have antifungal activity against some fungi in vitro [3,8], which can act synergistically with plant β-1,3-glucanases to inhibit fungal growth [9,10]. In recent studies, such antifungal activity of chitinases makes this protein an attractive candidate for the production of transgenic crop plants with increased expression of chitinase [11].

Induction of chitinase mRNAs by pathogen infection was detected in a variety of plants. The chitinase mRNAs encoding 26-, 27- and 30-kDa chitinases, respectively, were strongly induced in tomato plants by inoculation with Cladosporium fulvum [12]. Although the major putative role of chitinase is to defend against fungal pathogens,
chitinases were also induced by infections with *X. campestris* pv. *vesicatoria* [13], *Ralstonia* (*Pseudomonas*) *solanacearum* [14] and TMV [15].

In addition to a pathogen attack, exogenous application of chemicals and wounding have been found to activate or enhance biochemical defenses against potential pathogen attack. Class I tobacco chitinase CHN50 mRNA was shown to be induced by pathogen infection, salicylic acid, ethylene and wounding [16–19]. Acidic class III cucumber chitinase mRNA was strongly expressed by treatment with salicylic acid and 2,6-dichloronicotinic acid (INA) [20]. Treatment with benzothiadiazole induced the accumulation of acidic and basic class III tobacco chitinase mRNAs [21]. Class V chitinase mRNA homologous to bacterial exochitinases was found to be induced by TMV infection, ethephon, wounding and UV irradiation [22]. More recently, analysis of the chitinase gene structure including the promoter region suggests a close relationship between unique sequences in promoter region and expression of chitinase gene [19,23].

By using two-dimensional SDS polyacrylamide gel electrophoresis (PAGE), we have demonstrated previously that some PR proteins not detectable in healthy pepper plants were induced to accumulate by infection with *Phytophthora capsici* or *X. campestris* pv. *vesicatoria* [24,25]. In further studies, we reported differential induction and accumulation of chitinase isoforms in pepper plants inoculated with these pathogens or treated with mercuric chloride [2,13]. The roles of individual isoforms of chitinases were also evaluated in the compatible and incompatible interactions. More recently, we isolated and purified the chitinases and other chitin-binding proteins from pepper stems treated with mercuric chloride [3]. The purified chitinases contained high ratios of cysteine and glycine at the N-terminal chitin-binding domain and showed antifungal activity against some plant pathogenic fungi.

We have started to investigate the molecular expression of the chitinase gene family in pepper plants. In this paper, we report the isolation, sequencing, and expression analysis of CAChi2 cDNA clone encoding a basic class II chitinase in pepper plants. The gene was induced in pepper plants by infection with *X. campestris* pv. *vesicatoria* or *P. capsici*, treatment with ethephon and benzothiadiazole.

### 2. Materials and methods

#### 2.1. Plant, pathogens, and elicitors

Pepper (Capsicum annuum L.) cultivar Hanbyul was used in this study. Pepper seeds were sown in plastic trays (55 × 35 × 15 cm) containing sterilized soil, sand and compost (1:1:1, v/v/v). Seedlings at the two-leaf stage were transplanted to plastic pots (5 × 15 × 10 cm) containing the same soil mixture. Pepper plants were raised in a growth room under 16-h day illumination at 27 ± 2°C.

Virulent (Ds1) and avirulent (Bv5-4a) strains of *X. campestris* pv. *vesicatoria* were used in this study. Pepper plants were inoculated by vacuum-infiltrating the bacterial suspension into the abaxial side of the fully expanded leaves with an atomizer. Virulent (S197) and avirulent (CBS178.26) isolates of *P. capsici* were grown on oatmeal agar plates for 10 days. To induce sporulation, the plates were placed under fluorescent light at 28°C for 3 days. The mycelia and sporangia were incubated in sterile water for 1 h at 4°C and then 1 h at room temperature to release zoospores. A small quantity of sterile cotton soaked in a zoospore suspension (10⁵ zoospores ml⁻¹) of the two isolates was placed on the stems of pepper plants at the six-leaf stage wounded by making a 1-cm longitudinal slit.

Entire plants were sprayed with 10 mM ethephon (Amchem Products), 25 µM methyl jasmonate (Aldrich), 1000 µg ml⁻¹ DL-β-amino-η-butryic acid (BABA) (Sigma), 20 µg ml⁻¹ benzothiadiazole (BTH) (Novartis), or 5 mM salicylic acid (Sigma). Pepper plants treated with ethephon and methyl jasmonate were covered with vinyl bags. At various intervals after inoculation and elicitor treatment, pepper leaves or stems were harvested from the inoculated plants for RNA isolation.

#### 2.2. Construction of cDNA library

A leaf cDNA library was constructed using poly (A)⁺ mRNA from pepper (cv. Hanbyul) leaves infected with avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria*. Poly (A)⁺ mRNA was isolated from the total RNA recovered from an oligo (dT)-cellulose column to synthesize the double-stranded cDNA. Double-stranded cDNA was recovered and ligated into the λZAPII (Stratagene,
La Jolla, CA) and packaged in vitro into phage particles following the manufacturer’s instructions (Amersham).

2.3. Preparation of the chitinase-coding region pchit II

The coding region, named pchit II, of the class II pepper chitinase from pepper genomic DNA was amplified by polymerase chain reaction (PCR) using two primers that introduced restriction sites at the ends of the amplification products. The two primers, named CLASS2N and CLASS2C, were synthesized using a DNA synthesizer. The 5’ and 3’ primers were CLASS2N 5’-AACAACCC(A:T)GATTTAGT(A/G)GC-3’ and CLASS2C 5’-GTTCTTT(A/G)TGTGTTACAGT-3’, which included amino acid sequence alignments of the highly conserved regions of different plant chitinases, respectively (Fig. 2). Amplification of the chitinase-coding region pchit II by PCR was performed in a DNA thermal cycler (Perkin Elmer). The amplification reaction mixture of 20 μl consisted of 100 ng pepper genomic DNA, 10 pmol of the CLASS2N primer, 10 pmol of the CLASS2C primer, 3.0 mM NTP, and Taq DNA polymerase in 10 × PCR buffer. The DNA-denaturation step was set at 94°C for 5 min, the primer annealing step at 44°C for 45 s, and the primer extension step at 72°C for 2 min. The reaction was allowed to run for 40 cycles. The PCR-amplified DNAs were fractionated on a 1% low melting agarose gel (FMC Bioproducts, ME). Desired fragments were purified from the gel, cloned into pT7Blue vector and sequenced. The probe pchit II (the CLASS2N-CLASS2C probe) for screening pepper chitinase cDNAs was prepared from the recombinant plasmid by digesting with BamHI and HindIII.

2.4. Screening of cDNA library

To isolate full-length cDNA clones of pepper chitinases, the cDNA library in λZAP II was screened with the chitinase-coding region pchit II as a probe. The probe pchit II was labeled with digoxigenin (DIG) using a random primer labeling kit (Boehringer Mannheim). About 50 000 plaques of the cDNA library were transferred onto duplicate nylon membranes (Hybond N+, Amersham). The blots were hybridized for 18 h at 65°C with the DIG-labeled probe. Hybridization was performed in a solution containing 5 × SSC, 0.1% N-lauroylsarcosine, 0.02% SDS and 1% blocking reagent. Blots were washed twice at room temperature for 10 min in 2 × SSC and 0.1% SDS, and then twice at 65°C for 5 min in 0.1 × SSC and 0.1% SDS. The membranes were exposed to X-ray film for autoradiography.

2.5. Sequencing of cDNA and comparative analysis

DNA sequences of the pchit II and putative pepper chitinase cDNA CACHI2 were determined by the dideoxynucleotide chain termination method using Sequenase Version 2.0 (USB, Cleveland, OH). The deletion mutants of the CACHI2 cDNA clone were generated using Erase-A-Base kit (Promega, Madison, WI). DNA sequence data were assembled and analyzed using PC/Gene software and the BLAST and ORF finder network services at the National Center for Biotechnology Information [26]. DNA sequences were further analyzed using DNASTAR (DNASTAR, Madison, WI).

2.6. Genomic DNA isolation and Southern blotting

Genomic DNA was extracted from pepper leaves by the modified method of Kim et al. [27]. Fresh leaf tissue (2 g) was ground with liquid nitrogen in a mortar. Genomic DNA was extracted in an urea extraction buffer (42% urea, 3.125 mM NaCl, 50 mM Tris–HCl, pH 8.0, 20 mM EDTA, pH 8.0). The genomic DNA (10 μg) extracted was digested with EcoRI, BamHI, and HindIII, and fractionated by electrophoresis on a 0.8% agarose gel. The genomic DNA was transferred onto a nylon membrane by vacuum blotting. Hybridization was performed using the 32P-labeled CACHI2 cDNA as a probe [28].

2.7. RNA isolation and Northern blotting

Total RNA was isolated from pepper leaves and stems by the guanidine isothiocyanate and guanidine hydrochloride extraction buffers, respectively [29,30]. Plant materials were ground in liquid nitrogen and transferred into a 50-ml polypropylene tube. A mixture of 2 M sodium acetate (pH 4.0), water-saturated phenol, and chloroform–isoamyl alcohol mixture (49:1)
(1:100:2, v/v/v) was added to the homogenates. After centrifugation, the aqueous phase was mixed with 1 vol. isopropanol, and then placed at −20°C for at least 1 h to precipitate RNA. The pellet was dissolved in TNE buffer (50 mM Tris–HCl, pH 7.5, 10 mM EDTA, 0.5% SDS) and again precipitated by adjusting the solution to 2 M LiCl. After centrifugation, the RNA pellet was washed with 80% ethanol, dried and dissolved in DEPC-treated water. For Northern blot analysis, total RNA (20 μg) was separated on a denaturing 1.5% formaldehyde agarose gel. The RNA was transferred to Hybond N+ membrane (Amer sham), followed by UV cross-linking. Hybridization was performed overnight at 50°C in a solution containing 7% SDS, 50% formamide, 5× SSC, 2% blocking reagent, 50 mM sodium phosphate (pH 7.0), and 0.1% N-lauroylsarcosine. Membranes were washed twice with 2× SSC and 0.1% SDS at room temperature for 10 min, and then twice with 0.1× SSC and 0.1% SDS at 65°C for 5 min. Membranes were exposed to X-ray films.

3. Results

3.1. Isolation of a cDNA clone encoding a basic class II chitinase

Two primers, N-terminus CLASS2N and C-terminus CLASS2C, were designed and synthesized from the conserved regions of the class II chitinase sequences previously reported in many plant species. The polymerase chain reaction (PCR) procedures were performed to amplify the coding region of the class II pepper chitinase from pepper genomic DNA using the two primers synthesized. After the PCR amplification, a distinct DNA fragment was visualized on an agarose gel. The size of the DNA fragment amplified was similar to the size expected, approximately 310 bp, which corresponds to the coding region of the class II chitinases between the two primers. The fragment was cloned into pT7Blue cloning vector and sequenced. The amplified fragment, named pchit II, of the class II pepper chitinase was identified to be 308 bp (data not shown). The pchit II sequence showed 79, 76, and 71% identities to potato, tomato and tobacco class II chitinase, respectively.

The cDNA library, which was constructed from mRNAs in pepper leaves inoculated with the avirulent strain Bv5-4a of \textit{X. campestris pv. vesicatoria}, was screened with the PCR-derived clone pchit II. Among approximately 50,000 plaques screened, more than 150 plaques showed positive hybridizations with the pchit II probe at high stringency. Following plaque purification and in vivo excision of the pBluescript SK (−) recombinant plasmid from the λZAP II, the sizes of 15 clones were determined by agarose gel electrophoresis and Southern blot analysis (data not shown). The two cDNA clones of ca. 1.5 and 0.4 kb, which hybridized to the pchit II probe, were sequenced. However, the 1.5-kb cDNA clone pchit II 1-6 was confirmed to encode a putative basic chitinase of pepper in the sequence comparison by the BLAST program. The putative basic chitinase cDNA pchit II 1-6 was designated CAChi2.

3.2. Nucleotide and deduced amino acid sequences of CAChi2 cDNA

The complete nucleotide and deduced amino acid sequences of the CAChi2 cDNA encoding a putative basic chitinase of pepper revealed that the CAChi2 cDNA contains 1004 bp with a 759-nucleotide open reading frame (data not shown). The N-terminal 24 amino acids exhibit the characteristics of a signal peptide with a highly hydrophobic core and characteristic amino acid composition near the cleavage site [31]. The CAChi2 does not have cysteine-rich domains and a C-terminal extension signal sequence, which is necessary for vacuolar targeting of mature proteins [32].

The pepper CAChi2 open reading frame encodes a 253-amino acid protein with a predicted molecular mass of 27,768 Da and pI of 9.39. Molecular mass of mature CAChi2 protein is 25,161 Da. A search of protein databases with the deduced amino acid sequence using the Genetics Computer Group BLAST program revealed that the protein encoded by CAChi2 shares significant sequence identity with all other plant class II chitinases cDNAs. To examine similarities and differences in individual amino acid sequence positions, in Fig. 1, we aligned the deduced amino acid sequence of CAChi2 with five chitinases from other plant species. The alignment shows that CAChi2 has highly conserved regions in the catalytic domain with identical position and length, as
compared to the conserved regions in potato and tomato chitinases [12,33,34]. The high levels of sequence identity, from 69 to 86%, were found between the pepper chitinase CACHI2 and each of class II chitinases of potato, tomato, tobacco, and petunia. In contrast, a much lower identity was found between the pepper chitinase and other classes of plant chitinases (data not shown).

A phylogenetic analysis of the 15 deduced chitinase amino acid sequences in Genbank was used to compare the relationship of the chitinase encoded by pepper CACHI2 to other chitinases. As shown in Fig. 2, well-separated branches of plant chitinases can be identified in the phylogenetic tree. The deduced proteins of the CACHI2 chitinase cDNA clone falls into a class II chitinase group. Chitinases of each class are clustered together. Class II chitinase group including CACHI2 has a high amino acid sequence homology to class I chitinases, but sequence dissimilarities to class IV chitinases.

Fig. 1. Comparison of the amino acid sequences derived from pepper (C. annuum L.) CACHI2 cDNA with sequences from potato (Solanum tuberosum) ChtA2 (accession no. U49969, [33]), uncultivated tomato (Lycopersicon chilense) PCHIT28 (accession no. Q40114, [34]), tomato (Lycopersicon esculentum) 26 kDa chitinase (accession no. Z15141, [12]), tobacco (Nicotiana tabacum) PR-P (accession no. X51426, [35]), and petunia (Petunia hybrida) 25.1 kDa chitinase (accession no. X51427, [35]). Amino acids marked in black boxes indicate sequence identity. Gaps introduced for optimal alignment are indicated by dashes (–). Putative signal peptide cleavage site is represented by an arrow (¡). Two conserved regions present in other plant species, used for the primer design, are labeled by I and II. The numbers at the right refer to amino acid residue positions in the respective sequence.
Genomic DNA was digested with the restriction enzymes EcoRI, BamHI, and HindIII, respectively, for which there are no internal restriction sites in the sequence of CAChi2 cDNA. The completely digested DNA was subjected to Southern blot hybridization with the EcoRI–XhoI insert from the isolated chitinase cDNA clone CAChi2 as a probe (Fig. 3). The CAChi2 cDNA hybridized to one EcoRI band, one BamHI band and two HindIII bands. All of these band sizes are long enough to represent intact genes, suggesting that the CAChi2 chitinase in pepper genome is encoded by single or two copy genes.

3.4. Expression of CAChi2 mRNA upon infection with pathogens and treatment with abiotic elicitors

RNA gel blot analysis was performed to monitor the expression of the chitinase CAChi2 mRNA upon infection with either X. campestris pv. vesicatoria or P. capsici. No transcripts homologous to CAChi2 cDNA were detected in the healthy pepper leaves. In the compatible interactions of X. campestris pv. vesicatoria with pepper, accumulation of CAChi2 mRNA was considerably induced in the leaves by 30 h after inoculation (Fig. 4). In the incompatible interaction, the CAChi2 mRNA was first detected 12 h after inoculation, followed by a maximum in transcript levels at 30 h. P. capsici-infected pepper stems also showed different accumulation of CAChi2 transcripts in the compatible and incompatible interactions (Fig. 5). Induction of CAChi2 appeared in both interactions 1 day after inoculation. In the compatible interac-
Fig. 4. Northern blot analysis of chitinase (CACHi2) mRNA accumulation in pepper leaf tissue during compatible and incompatible interactions after infection by virulent strain Ds1 and avirulent strain Bv5-4a of Xanthomonas campestris pv. vesicatoria, respectively. (A) Northern blot analysis of CACHi2 mRNA at various times after inoculation. (B) Densitometric comparison of CACHi2 mRNA levels between compatible and incompatible interactions. As a control, each blot was hybridized with a 25S rRNA probe from Capsicum annuum. The Northern blot analysis was repeated three times with similar results. Vertical bars represent S.E.

Fig. 5. Northern blot analysis of chitinase (CACHi2) mRNA accumulation in pepper stem tissue during compatible and incompatible interactions after infection by virulent isolate S197 and avirulent isolate CBS178.26 of Phytophthora capsici, respectively. (A) Northern blot analysis of CACHi2 mRNA at various times after inoculation. ‘W’ indicates total RNA extracted from the wounded stem tissues 1 day after wounding. (B) Densitometric comparison of CACHi2 mRNA levels between compatible and incompatible interactions. As a control, each blot was hybridized with a 25S rRNA probe from Capsicum annuum. The Northern blot analysis was repeated three times with similar results. Vertical bars represent S.E.

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monate, benzothiadiazole and salicylic acid resulted in little accumulation of the transcripts in the leaves (Fig. 6A). Time course induction of CACHi2 transcripts by ethephon treatment is shown in Fig. 6B. The transcript level began to increase at 18 h after treatment with ethephon, with a maximum at 24 h. After application of ethephon, a chemical releasing gaseous ethylene, we examined whether or not hydrochloric acid (HCl) and phosphoric acid (H3PO3) as breakdown products of ethephon when applied to pepper plants induced transcripts of CACHi2. There was no induction of the transcript accumulation in pepper leaves by treatment with the two chemicals (data not shown). When treated with methyl jas-
Fig. 6. (A) Expression pattern of CACHI2 mRNAs in pepper leaves treated with ethephon, methyl jasmonate, DL-β-amino-n-butyrnic acid, benzothiadiazole, and salicylic acid. (B) Time course of induction of CACHI2 mRNA in pepper leaves treated with 10 mM ethephon. As a control, each blot was hybridized with a 25S rRNA probe from C. annuum. The Northern blot analysis was repeated three times with similar results.

4. Discussion

In this study, the pepper basic chitinase CACHI2 cDNA was isolated from the cDNA library of pepper leaves infected with X. campestris pv. vesicatoria, using a probe amplified from pepper genomic DNA as a template by PCR. The PCR product was amplified by the two primers, which were degenerated based on the conserved regions of class II chitinase amino acid sequences of different plant species previously reported [12,35,36]. The size of the CACHI2 clone was the same as those of some chitinase amino acid peptides such as tomato PCHIT28, tomato 26 kDa chitinase, and tobacco PR-P (Fig. 1). CACHI2 does not have a chitin-binding domain [37] and a C-terminal extension sequence [32], indicating that the CACHI2 belongs to the class II chitinase. The chitin-binding domain is known to be a major characteristic of class I chitinase [38]. Class I and II chitinases have leader sequences and catalytic regions as common features, whereas class II chitinase do not have a cysteine-rich chitin-binding domain, a hinge region, and a C-terminal extension sequence present in the class I chitinase [39]. Comparison of N-terminal amino acid sequences of basic pepper chitinases showed that the basic CACHI2 chitinase is not identical with the basic chitinases b1 (32 kDa, pI 9.0) and b2 (22 kDa, pI 9.1) previously purified from pepper plants [3], indicating the presence of another chitinase isoform in pepper plants. Cluster analysis of the deduced peptide of CACHI2 cDNA with the alignments of chitinases from 23 known chitinases also confirmed that CACHI2 can be classified into the class II chitinases (Fig. 2). With a tobacco class I chitinase CHN50, it has been suggested that some C-terminal extension sequences are necessary for targeting the protein to the vacuole [32,40] and that sequence changes in the vacuolar targeting peptide allow a gradual transition from vacuolar retention to secretion [40]. C-Terminal extension sequences were not found in the pepper CACHI2 chitinase, as in the petunia class II chitinase which has been found to be extracellularly located [35]. The calculated isoelectric point (pI) of CACHI2 is 9.39 that is not typical in the class II chitinases known to be usually more acidic proteins [38]. Although the CACHI2 chitinase gene may encode a basic chitinase, its localization seems to be extracellular, because of absence of a C-terminal extension signal. These suggestions are well supported by the findings of Kombrink et al. [41], which showed that potato basic chitinase was targeted to the extracellular space. Our recent observation that the activity of a basic chitinase isoform with pI 9.8, i.e. similar to that of CACHI2 chitinase, was induced in the intercellular washing fluid of X. campestris pv. vesicatoria-infected leaves [13] supports the possible extracellular localization of CACHI2 chitinase in pepper leaves.

The Southern blot analysis of the pepper chitinase gene indicated that the basic chitinase CACHI2 is encoded by one or two genes in pepper plants (Fig. 3). The fragment restricted with EcoRI or BamHI was hybridized as a single band, whereas the fragment restricted with HindIII was hybridized as double bands. These bands may
reflect either two chitinase gene copies differently located in the pepper chromosomes or a chitinase gene that has an internal cleavage site for *HindIII* in the intron region.

Interestingly, the CAChi2 chitinase mRNA increased more markedly in response to the avirulent strain than to the virulent strain of *X. campestris pv. vesicatoria*. Strong expression of the CAChi2 chitinase mRNA in the incompatible infection of *X. campestris pv. vesicatoria* may result in a high accumulation of basic chitinase activity in the intercellular space of pepper leaves, as recently demonstrated by Lee and Hwang [13]. In the incompatible interaction, the mRNA expression of CAChi2 chitinase greatly increased at 18–24 h, when a hypersensitive response, like rapid and localized tissue collapse, appeared against the avirulent strain infection. The great accumulation of the CAChi2 chitinase mRNA at 24–30 h after appearance of hypersensitive response may be significant in defense of pepper plants against *X. campestris pv. vesicatoria* infection. In contrast, mRNA induction of CAChi2 chitinase gene was relatively low until 30 h after inoculation with the virulent strain. At this time, no symptoms appeared on the pepper leaves. Chlorotic and susceptible lesions began to occur 3 days after inoculation with the virulent strain. *P. capsici* infection in pepper stems greatly induced the accumulation of the CAChi2 mRNA in the incompatible interaction rather than in the compatible interaction (Fig. 5), which suggested that the CAChi2 chitinase gene may be required for the expression of defense against *P. capsici* infection.

Application of ethephon (2-chloroethylphosphonic acid) induced a strong expression of CAChi2 mRNA in pepper leaves, whereas methyl jasmonate, BTH and salicylic acid induced little expression. Enhancement of the CAChi2 mRNA expression in pepper leaves upon treatment with ethylene-releasing ethephon suggests that expression of CAChi2 gene may be controlled by an ethylene-dependent signal transduction mechanism, as described recently for the PR-1 gene expression in pepper plants [42]. Recently, Tornero et al. [43] have demonstrated that ethephon, but not each of the breakdown products phosphonic acid and hydrochloric acid, induced PR-1 genes in tomato. Ethylene is thought to be the natural mediator of PR protein accumulation [44].

Taken together, it seems likely that in pepper defense- or pathogenesis-related plant responses, induction of CAChi2 mRNA is intrinsically associated with ethylene biosynthesis. However, further detailed studies will be necessary to precisely elucidate the role of ethylene in the induction of CAChi2 gene expression in pepper plants.

References

[12] N. Danhash, C.A.M. Wagemakers, J.A.L. van Kan, P.J.G.M. de Wit, Molecular characterization of four chitinase cDNAs obtained from *Cladosporium fulvum*-


