Expression of Peroxidase-like Genes, H₂O₂ Production, and Peroxidase Activity During the Hypersensitive Response to *Xanthomonas campestris* pv. *vesicatoria* in *Capsicum annuum*

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Pepper ascorbate peroxidase-like (*CAPOA1*), thioredoxin peroxidase-like (*CAPOT1*), and peroxidase-like (*CAPO1*) clones were isolated from pepper leaves inoculated with avirulent strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria*. *CAPOA1*, *CAPOT1*, and *CAPO1* mRNA disappeared 18 to 30 h after the bacterial infection when the hypersensitive response (HR) was visible. In contrast, peroxidase activity reached a peak at 18 h after infection and then declined at 24 and 30 h when H₂O₂ accumulation level was maximal. These results suggest that the striking accumulation of H₂O₂ and strong decrease in peroxidase activity during the programmed cell death may be due to the strong suppression of *CAPOA1*, *CAPOT1*, and *CAPO1* gene expression. Infection by *Phytophthora capsici* or *Colletotricum gloeosporioides* also induced the expression of the three putative peroxidase genes in pepper tissues. *CAPOA1* mRNAs were in situ localized in phloem areas of vascular bundles in pepper tissues infected by *Colletotricum coccodes*, *P. capsici*, or *C. gloeosporioides*. Exogenous treatment with H₂O₂ strongly induced the *CAPOA1* and *CAPOT1* transcription 1 h after treatment, while the *CAPO1* transcripts accumulated 12 h after H₂O₂ treatment. We suggest that pepper ascorbate peroxidase and thioredoxin peroxidase genes may function as regulators of H₂O₂ level and total peroxidase activity in the oxidative burst during the HR to incompatible pathogen interaction in pepper plant.

Striking increase of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), hydroxyl free radical (OH^{*}), and hydroperoxyl radical (HO₂) in plants has been demonstrated to occur in the early resistance response to plant pathogen attack (Mehdy 1994). ROS generation during plant-pathogen interactions was previously observed in potato tubers infected with *Phytophthora infestans* (Doke 1983). The striking release of ROS, termed the oxidative burst, is a rapid response of plant cells to elicitors and pathogens. Current research has been focused on elucidating the molecular machinery responsible for generation of ROS and the roles of ROS,

particularly H₂O₂, in regulating various aspects of disease resistance. H₂O₂ has not only been implicated in direct antimicrobial effects but also functions as a threshold trigger for the hypersensitive response (HR), a substrate for oxidative cross-linking of the plant cell wall less digestible by microbial enzymes, and a diffusible signal for induction of cellular protectant genes in surrounding cells (Baker and Orlandi 1995; Grant and Loake 2000; Lamb and Dixon 1997; Mehdy et al. 1996).

Various peroxidases were found to play significant roles in defense mechanisms and development of plants (Hiraga et al. 2001). In particular, ascorbate peroxidase is an antioxidant enzyme that removes H₂O₂ in plant oxidative defense systems induced by wounding, pathogen attack, and environmental stresses. In accordance with its role in scavenging H₂O₂, ascorbate peroxidase expression is induced in response to many environmental stresses that result in accumulation of ROS (Karpinski et al. 1997; Kubo et al. 1995; Mittler and Zilinskas 1992). It has been suggested that the inhibition of ascorbate peroxidase and catalase activities by salicylic acid during the response of plants to invading pathogens results in the accumulation of H₂O₂ and the activation and acceleration of programmed cell death (Chen et al. 1993; Durner and Klessig 1995; Rao et al. 1997). More recently, expression of an ascorbate peroxidase gene was found to be up-regulated in barley leaf tissues after inoculation with *Blumeria graminis* f. sp. *hordei* (Hückelhoven et al. 2001). The function of thioredoxin peroxidase, an antioxidant enzyme eliminating H₂O₂, which is related to plant defense upon pathogen attack, has not yet been described in plants. Peroxidases, generally called 'POX,' were shown to consist of a large number of isoenzymes in single plant species and to perform various functions (Hiraga et al. 2001). Since peroxidases are implicated in the deposition of cell-wall strengthening materials, such as lignin, suberin, and extensin, changes in peroxidase activity during resistant and susceptible interactions have also been monitored in a number of plant-pathogen interactions (Muthukrishnan 1999; Yi and Hwang 1998).

Differential hybridization has successfully been used to isolate differentially various defense-related genes expressed in pathogen-infected plants (Gadea et al. 1996; Jung and Hwang 2000a, 2000b). In the present study, we cloned *CAPOA1* (ascorbate peroxidase), *CAPOT1* (thioredoxin peroxidase), and *CAPO1* (peroxidase) genes from pepper leaves infected by avirulent strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria* using the differential hybridization technique. The roles of these three

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Nucleotide sequence data are available in the EMBL database under accession numbers AF442387 (*CAPOA1*), AF442385 (*CAPOT1*), and AF442386 (*CAPO1*).

putative peroxidase genes as H₂O₂ level regulators were studied in pepper defense against pathogen attack. The expression and regulation of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in response to fungal, oomycete, and bacterial pathogen infection and by H₂O₂ treatment was examined using Northern blot analysis, in situ hybridization technique, peroxidase activity assay, and endogenous H₂O₂ measurement.

RESULTS

Isolation and characterization of cDNAs encoding ascorbate peroxidase-like, thioredoxin peroxidase-like, and peroxidase-like genes.

The three pepper cDNA clones encoding different putative peroxidases were isolated from a cDNA library of pepper leaves inoculated with avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria*, using differential hybridization. The full-length peroxidases isolated were designated *CAPOA1* (*Capsicum annuum* ascorbate peroxidase, accession no. AF442387), *CAPOT1* (*Capsicum annuum* thioredoxin peroxidase, accession no. AF442385) and *CAPO1* (*Capsicum annuum* peroxidase, accession no. AF442386). Each sequence of the cDNA clones was analyzed and compared with the previously described sequences from other plant species.

The pepper *CAPOA1* cDNA clone is 1,138 bp, and the open reading frame (ORF) encodes a putative ascorbate peroxidase protein of 287 amino acids (Table 1). The poly(A) tail in the clone is preceded by the putative polyadenylation signal AA-GAAA. The predicted *CAPOA1* protein has a molecular mass of 31.71 kDa, isoelectric point (pI) of 7.74, and a potential N-glycosylation site (N-G-S) at position 56. The predicted amino acid sequence of *CAPOA1* was compared with ascorbate peroxidase from other plant species (Table 1). The putative protein encoded by *CAPOA1* was 66% identical to tobacco (*Nicotiana tabacum*) (accession no. AAA86689; Orvar and Ellis 1995), 67% identical to cucumber (*Cucumis sativus*) (accession no. T10189), 66% identical to tomato (*Lycopersicon esculentum*) (accession no. CAB58361; Gadea et al. 1999), and 66% identical to bell pepper (*Capsicum annuum*) (accession no. CAA57140; Schantz et al. 1995). *CAPOA1* had two essential histidine residues, a distal histidine (H-40) and a proximal histidine (H-160), known to be involved in peroxidase-specific catalysis (Fishel et al. 1991). R-L-A-W-H, a highly conserved

sequence around the distal histidine residues in the class I peroxidases (Poulos and Kraut 1987), was found in *CAPOA1*. The amino acid sequence near the proximal histidine of *CAPOA1* also was highly conserved. The conserved aspartate (D-205) residue may be related to peroxidase-specific catalysis, and tryptophan (W-176) may contribute to storage of oxidizing equivalents in compound I (Fishel et al. 1991). One cysteine residue (C-30) found to be important for intramolecular folding (Greenblatt et al. 1989) is conserved in the five ascorbate peroxidases listed in Table 1.

The *CAPOT1* cDNA clone contains an ORF of 723 bp, which encodes a putative thioredoxin peroxidase of 162 amino acids (Table 1). The predicted polyadenylation signal is AATAAT, and there is no potential N-glycosylation site in the nucleotide sequence. The protein encoded by *CAPOT1* has a molecular mass of 17.4 kDa and pI of 6.08. A protein database search with the deduced amino acid sequence revealed an extensive homology of *CAPOT1* to plant thioredoxin peroxidases (Table 1). *CAPOT1* showed 81% identity to Arabidopsis (*Arabidopsis thaliana*) (accession no. AAD28242) and 81% identity to Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) (accession no. AAD33602; Choi et al. 1999). In addition, thio-

Fig. 1. Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in various pepper organs. Total RNA (20 µg) from each sample was loaded in each lane of 7.4% formaldehyde agarose gel. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA (rRNA).

Table 1. Characteristics of full-length cDNAs encoding ascorbate peroxidase, thioredoxin peroxidase, and peroxidase isolated from pepper leaves infected by avirulent strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria*

Protein	5'-UTR (bp)	Coding region (bp)	3'-UTR (bp)	Amino acids Mass pI	Conserved protein motif	Sequences with high identities		
						Origin	Accession no.	Identity (%)
<i>CAPOA1</i> (AF442387) Ascorbate peroxidase	23	861	254	287 31.71 kDa 7.74	APIMRLRLAWHDA ^a DIVALSGGHTL ^b	tobacco	AAA86689	66
						cucumber	T10189	67
						tomato	CAB58361	66
						bell pepper	CAA57140	66
<i>CAPOT1</i> (AF442385) Thioredoxin peroxidase	51	486	186	162 17.4 kDa 6.08	VPGAFPTPTCKHVPGF IEKA EILCVSVNDPFFVMKAW	Arabidopsis	AAD28242	81
						Chinese cabbage	AAD33602	81
						rice	AAG40130	75
<i>CAPO1</i> (AF442386) Peroxidase	61	996	165	332 36.07 kDa 8.8	HFHDCFVQGCDASIL ^c VSCADILALAARDSV ^d LVTLVGGHTIGTSA ^b	tomato	T07008	89
						peanut	P22196	73
						Arabidopsis	11513747	49
						alfalfa	JC4781	43

^a peroxidase active site.

^b peroxidase proximal heme-binding region.

^c peroxidase distal heme-binding region.

^d central conserved region.

redoxin peroxidase (accession no. AAG40130) of rice (*Oryza sativa*) is 75% identical to CAPOT1. The three histidines (H-30, H-55, and H-97) are conserved in CAPOT1. CAPOT1 also contains the two conserved putative active site cysteine residues (C-51 and C-76), which indicated that CAPOT1 belongs to the 2Cys-Prx protein subfamily (Choi et al. 1999).

CAPO1, encoding a putative peroxidase, consists of 1,222 bp with a putative polyadenylation sequence of AATAAA (Table 1). The ORF encodes a putative protein of 332 amino acid residues. An N-glycosylation site is predicted at position 214. The estimated molecular mass of the mature polypeptide of CAPO1 is 36.1 kDa, and the pI is 8.88 (Table 1). The deduced amino acid sequence of CAPO1 was compared with other plant peroxidases. CAPO1 is 89% identical with tomato (*Lycopersicon esculentum*) defense-related peroxidase

(accession no. T07008; Gadea et al. 1996), 73% homologous with peanut (*Arachis hypogaea*) cationic peroxidase (accession no. P22196; Buffard et al. 1990), 49% homologous with Arabidopsis (*Arabidopsis thaliana*) lignification-related peroxidase (accession no. 11513747; Ostergaard et al. 2000), and 43% homologous with alfalfa (*Medicago sativa*) pathogen-induced peroxidase (accession no. JC4781; el-Turk et al. 1996) (Table 1). CAPO1 has three conserved histidine residues (H-72, H-74, and H-196), one of them involved in the acid/base catalysis at position 74 and the other one involved in heme stabilization at position 196 (Welinder 1992). In addition, eight conserved cysteines (C-43, C-76, C-81, C-119, C-125, C-213, C-245, and C-328), which are a characteristic of class II and III peroxidases, are present in CAPO1 and may form disulfide bridges (Welinder 1992). Aspartate

Fig. 2. Time courses of **A**, total peroxidase activity, **B**, H₂O₂ levels, and **C**, CAPOA1, CAPOT1, and CAPO1 expression in pepper leaf tissues after inoculation with virulent (Ds1) and avirulent (Bv5-4a) strains of *Xanthomonas campestris* pv. *vesicatoria* at the six-leaf stage. The data are the mean values $\pm$ standard deviations of three replicates. Total RNA (20 μ g) from each sample was loaded in each lane of 7.4 % formaldehyde agarose gel. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA (rRNA). Mock = mock-inoculated leaf tissues.

(D-127) and arginine (R-152) residues were predicted to be involved in salt bridge formation (Welinder 1985).

Differential expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in various pepper organs.

Northern blot analyses were performed to examine organ-specific expression of *CAPOA1*, *CAPOT1*, and *CAPO1* in pepper leaf, stem, root, flower, and green and red fruit (Fig. 1). *CAPOA1* gene was most highly expressed in red fruits. A slight *CAPO1* transcription occurred only in root tissue. Unlike *CAPOA1* and *CAPO1*, *CAPOT1* was expressed in all pepper organs, with the highest expression in stem tissue and the lowest in red fruit.

Peroxidase activity and endogenous H_2O_2 accumulation in bacteria-infected leaf tissue.

Time courses of total peroxidase activity were determined in pepper leaf tissues inoculated with avirulent strain Bv5-4a and virulent strain Ds1 of *X. campestris* pv. *vesicatoria* (Fig. 2A). In the incompatible interaction, enzyme activity was detected at 12 h after bacterial infection, reaching a peak in the leaf tissues at 18 h when the HR was visible at the infected sites. Thereafter, the level of peroxidase activity rapidly decreased in the heavily infected leaves. In the compatible interaction, peroxidase activity gradually increased in leaves inoculated with the virulent strain Ds1. The enzyme activity level at 18 h after inoculation in the incompatible interaction was approximately 3.5-fold higher than that in the compatible interaction. In contrast, the peroxidase activity at 30 h after inoculation in the compatible interaction was twofold higher than that in the incompatible interaction. These results indicated that the increase in total peroxidase activity was higher at earlier times after inoculation in the incompatible interaction than in the compatible interaction.

The accumulation of ROS such as H_2O_2 is a typical characteristic of plant tissues undergoing HR (Lamb and Dixon 1997). The H_2O_2 levels in leaf tissues were monitored at various time intervals after inoculation with avirulent strain Bv5-4a and virulent strain Ds1 of *X. campestris* pv. *vesicatoria* (Fig. 2B). No significant changes occurred in uninoculated leaf tissues. The first oxidative burst was observed at 2 h after infection in the incompatible and compatible interactions, and the levels of H_2O_2 were similar. However, a second striking oxidative burst, namely a maximal accumulation of H_2O_2 that was sixfold more than in the compatible interaction, occurred only in the incompatible interaction at 24 h after inoculation, when HR was visible at the inoculated sites. In contrast, low levels of H_2O_2 remained constants in the compatible interaction from 18 to 30 h after inoculation.

Effect of exogenous H_2O_2 on expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes.

The expression of *CAPOA1*, *CAPOT1*, and *CAPO1* was examined in pepper leaves treated with H_2O_2 (Fig. 3). The three peroxidase-like genes were strongly induced at different time intervals after H_2O_2 treatment with various concentrations of H_2O_2 . The putative ascorbate peroxidase *CAPOA1* gene and thioredoxin peroxidase *CAPOT1* gene began to be expressed within 15 min and 1 h after treatment with H_2O_2 , respectively, whereas the initial transcription of the putative peroxidase *CAPO1* was detected at 12 h after H_2O_2 treatment.

Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes after pathogen infection.

The transcription of *CAPOA1*, *CAPOT1*, and *CAPO1* in pepper leaf, stem, and fruit tissues in response to pathogen infection was examined using *X. campestris* pv. *vesicatoria*, *P. capsici*, and *C. gloeosporioides* (Figs. 2C and 4).

CAPOA1 and *CAPO1* were rapidly and strongly induced in leaf tissues by *X. campestris* pv. *vesicatoria*, irrespective of compatible or incompatible interaction (Fig. 2C). In contrast, *CAPOT1* was expressed in the uninoculated and inoculated leaf tissues. Interestingly, mRNA levels of the three peroxidase-like genes did not increase at 18 to 30 h when the hypersensitive reactions appeared in leaves infected by the avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria* (Fig. 2C).

Infection by *P. capsici* induced *CAPOA1* and *CAPO1* more strongly in the incompatible interaction than in the compatible interaction (Fig. 4A). An interesting phenomenon was that the mRNA level of *CAPOT1* drastically declined in stem tissues upon oomycete infection, completely vanishing at 48 h after infection. Slight transcription of *CAPO1* was also detected in the compatible interaction. The time course inductions of *CAPOA1*, *CAPOT1*, and *CAPO1* were examined in green and red fruits infected with *C. gloeosporioides* (Fig. 4B). The three putative peroxidases were strongly induced in the green fruit by anthracnose disease. In contrast, they were not highly induced in the infected red fruit tissues, which were highly resistant to anthracnose disease (Lee et al. 2000a).

In situ localization of *CAPOA1* mRNA in infected pepper tissues.

To examine in situ localization of *CAPOA1* mRNA in pepper leaf, stem, and fruit tissues infected by pathogens, in situ hybridization was performed using a digoxigenin (DIG)-labeled *CAPOA1* cDNA as a probe or soybean peroxidase *GMIPER1* cDNA as a control probe (Fig. 5). In situ hybridization with a soybean peroxidase *GMIPER1* DIG-labeled cDNA probe resulted in no labeling of healthy and infected pepper tissues (data not shown). No hybridization signal of the *CAPOA1*

Fig. 3. Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in pepper leaves in response to H_2O_2 at the six-leaf stage. Time course analysis was performed after treatment with 10 mM concentration of H_2O_2 . The analyses of concentration effect were performed at 18 h after H_2O_2 treatment at various concentrations. Total RNA (20 μ g) from each sample was loaded in each lane of 7.4% formaldehyde agarose gel. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA (rRNA). Mock = mock-treated leaves.

cDNA probe was detected in uninfected, healthy leaf tissues (Fig. 5A). High levels of expression of *CAPOA1* mRNA occurred in the phloem area of the vascular bundle in *C. coccoodes*-infected pepper tissues at the four-leaf (Fig. 5B) and eight-leaf stages (Fig. 5C). Stem tissue samples obtained at 36 h after infection by virulent isolate S197 and avirulent strain CBS178.26 of *P. capsici* were used for in situ hybridization analysis. The uninfected, healthy stem tissues were almost free of labeling (Fig. 5D). However, distinct accumulation of *CAPOA1* mRNA was observed in the phloem area of vascular bundles in the stem tissues infected by both avirulent and virulent strains of *P. capsici* (Fig. 5E and F). The *CAPOA1* mRNA was intensively localized in phloem cells of green and red fruit tissues infected by *C. gloeosporioides* (Fig. 5H and I). However, no hybridization signal was observed in the uninfected, healthy green fruit tissue (Fig. 5G).

Effect of salicylic acid on expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes.

The three pepper peroxidase-like genes *CAPOA1*, *CAPOT1*, and *CAPO1* were differentially responsive to salicylic acid (SA) in leaves (Fig. 6). *CAPOA1* mRNA began to be induced 6 h after SA treatment and was highly increased at 18 to 24 h after treatment. However, the level of *CAPOT1* mRNA, constitutively expressed in untreated pepper leaves, was gradually de-

creased by 24 h after SA treatment. *CAPO1* transcription also was activated by SA, but the induction was much weaker than that of *CAPOA1*. Low levels of *CAPO1* expression were detected 6 h after SA treatment, and a little transcript accumulation occurred in leaf tissue 24 h after treatment.

DISCUSSION

We isolated three different peroxidase gene homologs from pepper leaves infected with the avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria*. The three genes encode a putative ascorbate peroxidase (*CAPOA1*), a putative thioredoxin peroxidase (*CAPOT1*), and a putative peroxidase (*CAPO1*). Because these peroxidase gene homologs were isolated from pepper leaves showing HR to the avirulent bacterial infection, it was suggested that the predicted peroxidases *CAPOA1*, *CAPOT1*, and *CAPO1* may be involved in pepper defense against pathogen attack.

Functions of H_2O_2 as a ROS in oxidative burst in response to bacterial infection.

In our present study to determine whether or not oxidative burst occurs in pepper-pathogen interactions, we measured the H_2O_2 endogenously generated at various times in pepper plant infected by *X. campestris* pv. *vesicatoria*. A minor oxidative

Fig. 4. Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in pepper stem and fruit tissues infected with pathogens. **A**, *CAPOA1*, *CAPOT1*, and *CAPO1* expression in pepper stems after infection by virulent isolate S197 and avirulent isolate CBS178.26 of *Phytophthora capsici* at the four-leaf stage. **B**, *CAPOA1*, *CAPOT1*, and *CAPO1* expression in pepper green and red fruits at various time intervals after infection by *Colletotrichum gloeosporioides*. Total RNA (20 µg) from each sample was loaded in each lane of 7.4% formaldehyde agarose gel. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA (rRNA). Mock = mock-inoculated tissues.

burst occurred between 2 and 6 h after inoculation in the compatible and incompatible interactions, while a major intensive oxidative burst at 18 h after inoculation was generated only in the incompatible interaction (Fig. 2B). The major oxidative burst occurred with a maximal H_2O_2 level at 24 h when HR was visible in inoculated plant tissues, which suggests that the major oxidative burst in the incompatible interaction may be closely related to the resistance response of pepper to *X. campestris* pv. *vesicatoria* infection. The biphasic accumulation of H_2O_2 during the plant oxidative burst has been found in other plant-pathogen interactions (Baker et al. 1991; Keppler et al. 1989; Lamb and Dixon 1997). Able and associates (2000) demonstrated that, in resistant tobacco cells, H_2O_2 was produced in a first oxidative burst between 0 and 2 h and in a second more intensive oxidative burst between 8 and 10 h after inoculation with the incompatible race of *P. nicotianae*.

Effects of H_2O_2 on induction of *CAPOA1*, *CAPOT1*, and *CAPO1* were examined in pepper leaves at various time intervals after exogenous treatment with H_2O_2 at various concentrations. *CAPOA1* and *CAPOT1* were induced early, within 1 h after H_2O_2 treatment, whereas the initial accumulation of *CAPO1* transcripts occurred at 12 h after treatment with H_2O_2 (Fig. 3). These results suggest that *CAPOA1* and *CAPOT1* genes may be expressed early for detoxifying excessive H_2O_2 . *CAPO1* may also be induced by H_2O_2 as a signal molecule, as are other defense-related genes. In addition, a low concentration, 10 μM H_2O_2 , was sufficient for inducing the expression of *CAPOA1*, *CAPOT1*, and *CAPO1* (Fig. 3). Several genes encoding glutathione S-transferase, glutathione peroxidase, and polyubiquitin, which are involved in cellular protection, were

demonstrated to be activated by exogenous H_2O_2 from the oxidative burst during the HR in soybean cells (Levine et al. 1994). Morita and associates (1999) reported that the transcript level of cytosolic ascorbate peroxidase was dramatically increased in the suspension cultures of rice embryos treated with H_2O_2 .

Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes upon plant pathogen infection.

The transcripts of the three peroxidase-like genes *CAPOA1*, *CAPOT1*, and *CAPO1* did not accumulate at 18 to 30 h when the hypersensitive reactions appeared in the pepper leaves infected by the avirulent strain Bv5-4a of *X. campestris* pv. *vesicatoria* (Fig. 2C). Peroxidase activity reached a peak in leaves at 18 h after inoculation of avirulent strain Bv5-4a and drastically declined thereafter. In the compatible interaction, peroxidase activity was maintained at a low level to 24 h and dramatically increased at 30 h after infection (Fig. 2A). Therefore, at 18 h, when transcription of the three peroxidase genes declined, peroxidase activity started to decline, while the hypersensitive reaction was intensively progressing. Moreover, unlike that in the compatible interaction, H_2O_2 level in the incompatible interaction started to rise at 18 h, and the maximal H_2O_2 accumulation was detected at 24 h and 30 h after inoculation (Fig. 2B). Therefore, it was suggested that the striking increase of H_2O_2 at the time when cell death progressed drastically may be due to the strong suppression of *CAPOA1*, *CAPOT1*, and *CAPO1* gene expression. While environmental stresses allow the production of ROS to pose a danger to cells, ROS-scavenging mechanisms are induced to remove ROS and

Fig. 5. In situ localization of *CAPOA1* mRNA in pepper leaf, stem, and fruit tissues infected by pathogens. **A**, Uninoculated leaf tissues at the eight-leaf stage. **B**, and **C**, Four- and eight-stage leaf tissues at 24 h after infection with *Colletotrichum coccodes*, respectively. **D**, Uninoculated stem tissues at the four-leaf stage. **E**, and **F**, Stem tissues at 36 h after infection with virulent isolate S197 and avirulent isolate CBS178.26 of *Phytophthora capsici* at the four-leaf stage, respectively. **G**, Uninoculated red fruit tissues. **H**, and **I**, Green and red fruit tissues at 48 h after infection by *Colletotrichum gloeosporioides*, respectively. Each sample was hybridized with digoxigenin-labeled *CAPOA1* cDNA. P = phloem; X = xylem. Bars = 30 μm .

to protect host cells. However, when ROS production is enhanced to trigger the HR, the toxicity of ROS or ROS-derived compounds may contribute to host cell death (Adam et al. 1989; Keppler and Baker 1989). One mechanism suggested to sustain ROS levels during the oxidative burst is the inhibition of ascorbate peroxidase, which was observed after addition of a race-specific fungal elicitor to enriched plasma membrane fractions from tomato (Vera-Estrella et al. 1992). The translation of mRNA encoding cytosolic ascorbate peroxidase also was found to be inhibited during the HR in tobacco (Mittler et al. 1998).

In general, SA content was increased in plant tissues infected by various pathogens (Martinez et al. 2000). Expression of many defense-related genes is regulated by an SA-dependent pathway (Ward et al. 1991). Exogenous application of SA could trigger induction of defense-related genes, resulting in systemic acquired resistance against a broad range of pathogens (Ryals et al. 1996). The three pepper peroxidase-like genes, *CAPOA1*, *CAPOT1*, and *CAPO1*, were also up- or down-regulated by SA treatment (Fig. 6). In particular, transcription of the putative ascorbate peroxidase *CAPOA1* gene was strongly activated in pepper leaves by SA treatment at 18 to 24 h when the HR occurred in the incompatible interaction with *X. campestris* pv. *vesicatoria* (Fig. 6), suggesting the involvement of *CAPOA1* in the triggering of HR. The potential of SA produced during the HR to inactivate catalase and ascorbate peroxidase activities also was demonstrated to contribute to the enhancement of HR cell death (Chen et al. 1993; Dietrich et al. 1997; Durner and Klessig 1996). In our study, the putative thioredoxin peroxidase *CAPOT1* was continuously expressed in all pepper organs (Fig. 1), but exogenous SA treatment of pepper leaves resulted in suppression of *CAPOT1* gene expression (Fig. 6). Interestingly, it has recently been demonstrated that thioredoxin peroxidase inhibits p53-induced apoptosis in mammalian cells (Zhou et al. 2000). One important aspect of p53 function in mammalian cells involves induction of apoptosis, likely mediated by redox. In their study, overexpression of thioredoxin peroxidase prevented the p53-dependent generation of ROS. Our results did not provide direct evidence to determine whether or not the oxidative burst is a direct cause of HR in the incompatible interaction of pepper with *X. campestris* pv. *vesicatoria*. However, H₂O₂ accumulation may play a significant role following the HR, because the HR was already clearly visible at 18 h after inoculation, when the major oxidative burst started. In contrast, it is interesting to note that the strong induction of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in the compatible interaction is effective in suppressing the accumulation of H₂O₂ during the disease development (Fig. 2).

Fig. 6. Expression of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in pepper leaves in response to 5 mM salicylic acid (SA) at the six-leaf stage. Time course analysis was performed after treatment with a 5 mM concentration of SA. Total RNA (20 µg) from each sample was loaded in each lane of 7.4% formaldehyde agarose gel. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA (rRNA). Mock = mock-treated leaf tissues.

The strong accumulation of *CAPOA1*, *CAPOT1*, and *CAPO1* transcripts in the incompatible interaction was induced in the pepper leaves infected by *P. capsici* (Fig. 4A), which suggests that the peroxidase gene expression may be related to ROS-associated defense response of pepper to *P. capsici* infection. An interesting fact is that the expression of *CAPOT1* in healthy stem tissues dramatically declined and disappeared at 48 h after infection. However, the role of H₂O₂ in the induced transcription of *CAPOA1*, *CAPOT1*, and *CAPO1* genes in incompatible interactions of *P. capsici* with pepper stems remains to be elucidated. In the green and red fruits inoculated with *C. gloeosporioides*, the three peroxidase genes were strongly activated in the green fruit by anthracnose disease (Lee et al. 2000a) but not in red fruits resistant to anthracnose disease. These results suggest that the pepper peroxidase genes may be inducible during the pathogenesis of anthracnose disease rather than in the disease resistance response.

In situ hybridization analysis revealed that *CAPOA1* transcripts were mainly localized in phloem areas of vascular bundles in pepper leaf, stem, or fruit tissues infected with *C. coccodes*, *P. capsici*, and *C. gloeosporioides* (Fig. 5). In our previous studies, the vascular bundle was considered an active area for high accumulation of transcripts of defense-related genes, i.e., PR-1, chitinase, and thionin in pepper leaf and stem tissues (Lee et al. 2000a, 2000b, 2000c). These earlier results are consistent with the findings that *CAPOA1* was actively induced in the phloem cells by various pathogen infections. Some PR-protein transcripts from other plants were found to be localized in the vascular bundle (Breda et al. 1996; Büchter et al. 1997; Eyal et al. 1993).

Northern blot analysis, in situ hybridization, peroxidase activity assay, and endogenous H₂O₂ measurement in the present study suggest that expression of *CAPOA1*, *CAPOT1*, and *CAPO1* in pepper-pathogen interactions may be closely correlated with H₂O₂ accumulation and peroxidase activity from the oxidative burst during the HR and programmed cell death or disease development in pepper plants. Despite the strong correlative evidence for the involvement of peroxidase-like genes in pepper resistance to pathogens, a specific role and regulation mechanism for each peroxidase has not been elucidated. Further study of functions, regulation, and signaling pathways of *CAPOA1*, *CAPOT1*, and *CAPO1* is required to gain an insight into defense and pathogenesis in pepper.

MATERIALS AND METHODS

Plant material.

Pepper seeds (*Capsicum annuum* L. cv. Hanbyul) were sown in a plastic tray (55 × 35 × 15 cm) containing a steam-sterilized soil mix of a commercial compost soil (peat moss, perlite, and vermiculite, 5:3:2, vol/vol/vol) and loam soil (1:1, vol/vol). Seedling plants (6) at the two-leaf stage were transplanted to plastic pots (5 × 15 × 10 cm). Plants were grown under controlled environmental conditions at 27 ± 1°C with 60 to 70% relative humidity and light intensity of 70 µmol photons/m²/s (white fluorescent lamps) for 16 h a day.

Pathogens and inoculation.

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. Pepper plants at the six-leaf stage were inoculated by vacuum-infiltrating bacterial suspension (10⁸ CFU/ml) into the abaxial side of fully expanded leaves (Kim and Hwang 2000). Bacteria-infected plants were kept in a humid chamber at 28°C with 100% relative humidity for 18 h. The plants were then placed in a growth room.

Isolates S197 and CBS178.26 of *Phytophthora capsici*, which were virulent and avirulent to the pepper cultivar Hanbyul, respectively, were cultured on oatmeal agar plates at 28°C for 7 days. The stems of pepper plants at the four-leaf stage were inoculated by covering the bottom region of stems with sterile cotton soaked in zoospore suspension (10^5 zoospores/ml). After inoculation, the plants were incubated in a growth room.

The isolate CHC2 of *Colletotrichum gloeosporioides* was cultured on potato dextrose agar plates in the dark for 5 days at 28°C. Conidial suspensions (10^6 conidia/ml) were prepared and were inoculated by dropping 40 µl of conidial suspension on mature green and red fruits. Mock-infected pepper fruits were treated with sterile tap water. The inoculated pepper fruits were incubated in a humid box at 28°C for 48 h. Infected regions of green and red fruit were harvested at various time intervals after inoculation.

Inocula of the isolate 2-25 of *Colletotrichum coccodes* were prepared for inoculation, as previously described by Hong and Hwang (1998). Pepper plants at the four- and eight-leaf stages were inoculated with the conidial suspension (10^6 conidia/ml) using the foliar-spray method. Inoculated pepper plants were incubated in a humid chamber for 36 h at 28°C and then moved to a growth room.

The leaves, stems, and fruits were sampled at various times after inoculation, were frozen in liquid nitrogen, and were stored at -70°C.

Isolation and sequence analysis of peroxidase cDNAs.

A cDNA library was constructed with 5 µg poly(A)⁺ mRNA of pepper leaves infected with *X. campestris* pv. *vesicatoria* (Kim and Hwang 2000). To isolate pathogenesis-related cDNA genes from the pepper cDNA library, differential hybridization was performed (Jung and Hwang 2000a). DIG-labeled probes were prepared from total mRNA of healthy and avirulent strain Bv5-4a-infected pepper leaves at 2, 6, 12, and 18 h after inoculation using reverse transcription-polymerase chain reaction (RT-PCR). Hybridization signals were detected following the procedures described by suppliers (Boehringer, Mannheim, Germany). The cDNA clones strongly expressed only in bacteria-inoculated leaves were selected. The selected cDNAs were sequenced on the ABI 310 DNA sequencer (Applied Biosystems Inc., Foster City, CA, U.S.A.) using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Inc., Foster City, CA, U.S.A.) according to manufacturer's instructions. Sequence data obtained from the cDNAs were analyzed using the PC/Gene software system (Intelligenetics, Inc., University of Geneva, Switzerland). The DNA sequences were compared with other sequences in the databases of GenBank using BLAST network services at the National Center for Biotechnology Information (Altschul et al. 1990).

RNA blot analysis.

Total RNA was prepared from pepper leaves, stems, roots, flowers, and fruits using the guanidine isothiocyanate method (Chomczynski and Sacchi 1987). Total RNA (20 µg) were separated by electrophoresis on a 7.4% formaldehyde agarose gel and were transferred to nylon membranes (Hybond-N⁺, Amersham, Little Chalfont, U.K.) (Sambrook et al. 1989). Putative ascorbate peroxidase *CAPOA1*, putative thioredoxin peroxidase *CAPOT1*, or putative peroxidase *CAPO1* cDNA probes were ³²P-labeled using a random priming kit (Boehringer Mannheim). Hybridization was performed overnight at 65°C in 5% (wt/vol) dextran sulfate, 0.25 M disodium phosphate, pH 7.2, 7% (wt/vol) sodium dodecyl sulfate (SDS), and 1 mM ethylenediamine tetraacetic acid (EDTA). The putative ascorbate peroxidase, thioredoxin peroxidase, and peroxi-

dase genes are all members of significant gene families. Therefore, it seemed likely that the hybridization signals might represent expression of more than one closely related family member. Equal loading of RNAs was confirmed by monitoring the levels of ribosomal RNA. RNA gel blot analyses of all samples were repeated three times with similar results.

In situ hybridization.

In situ hybridization of mRNA in pepper tissues was performed according to the method of Lee and associates (2000a, 2000b, 2000c). Pepper tissues embedded in paraplast were sectioned (10 µm in thickness) and were mounted on microscopic slides coated with poly-L-lysine (Sigma, St. Louis). After deparaffinization, the sections were treated with proteinase K (5 mg/ml) in 50 mM EDTA and 100 mM Tris-HCl, pH 8.0, for 30 min at 37°C. Prior to hybridization, sections were acetylated with 0.25% acetic anhydride in 100 mM triethanolamine, pH 8.0, for 10 min at room temperature. The sections were then hybridized with DIG-labeled *CAPOA1* cDNA probe using a Dig High prime kit (Boehringer) overnight at 42°C in a hybridization buffer. The hybridization signals were detected with the diluted nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, following the manufacturer's instructions (Boehringer). To demonstrate the specificity of the in situ hybridization, control tests were performed on healthy or inoculated tissues with soybean peroxidase *GMIPER1* cDNA (accession no. AF007211) (Yi and Hwang 1998) or without DIG-labeled probes.

Salicylic acid and H₂O₂ treatments.

To examine the gene induction by salicylic acid and H₂O₂ treatments, 5 mM of salicylic acid and H₂O₂ at 0.001, 0.01, 0.1, 1, 5, and 10 mM was sprayed onto or infiltrated into the leaves of pepper plants at the six-leaf stage, respectively. Treated pepper plants were incubated under standard conditions as described above. Pepper leaves were sampled at 18 h after SA and H₂O₂ treatments. Pepper leaves treated with 5 mM SA and 10 mM H₂O₂ were also sampled at various time intervals. After freezing in liquid nitrogen, leaf samples were stored at -70°C until use.

Peroxidase activity assay.

To extract total protein from pepper leaves inoculated with the two *X. campestris* pv. *vesicatoria* strains Ds1 and Bv5-4a at the six-leaf stage, frozen leaves (1 g) from three samples were crushed to a fine powder with a mortar and pestle in liquid nitrogen. Tissue was homogenized in 10 volumes per unit fresh weight of 10 mM sodium phosphate, 1% sodium metabisulphite, pH 6.0. Homogenates were centrifuged at $13,000 \times g$ at 4°C for 30 min, and aliquots of the supernatant were frozen at -70°C. Peroxidase activity assays were carried out according to the method of Hammerschmidt and associates (1982). Total peroxidase activity of crude extracts was spectrophotometrically measured at 470 nm, using guaiacol as the hydrogen donor. The reaction mixture was composed of 2.9 ml phosphate buffer (0.01 M, pH 6.0, 25°C), containing 1.25% (vol/vol) guaiacol and 0.1 M H₂O₂. The enzyme reaction was started by adding 100 µl of enzyme extracts, and the absorbance at 470 nm was recorded for 5 min at 25°C. Crude enzyme extracts were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units per min. Peroxidase activity of crude extracts was calculated using the molar extinction coefficient of tetraguaiacol (26.6×10^3 mol/cm), and enzyme activity was expressed as nano katal per mg of total proteins. The experiment was repeated twice, with similar results, and each experiment had three replications. Data presented are from one experiment only.

H₂O₂ analysis.

H₂O₂ was extracted from pepper leaf tissues at various time intervals after inoculation with *X. campestris* pv. *vesicatoria* Ds1 and Bv5-4a strains at the six-leaf stage, following the method of Okuda and associates (1991). Leaf material (0.5 g) from three samples was ground with a mortar and pestle in liquid nitrogen and 5 ml of 0.2 M HClO₄. The homogenate was centrifuged at 12,000 × g for 10 min. The supernatant was neutralized to pH 7.5 with 4 M KOH and was centrifuged at 1,000 × g for 1 min. An aliquot (200 µl) of the supernatant was applied to an anion-exchange column (1 ml) filled with AG1×8 resin (Bio-Rad Laboratory, Hercules, CA, U.S.A.). The column was eluted with 800 µl of distilled water. The eluate was used for the assay of H₂O₂. H₂O₂ was determined by a spectrophotometric assay (Okuda et al. 1991). The reaction mixture contained 1 ml of the eluate, 400 µl of 12.5 mM 3-dimethylaminobenzoic acid (DMAB) in 375 mM phosphate buffer (pH 6.5), 80 µl of 1.3 mM 3-methyl-2-benzothiazoline hydrazone (MBTH), and 20 µl of peroxidase (0.25 unit) in a total volume of 1.5 ml. The reaction was started by the addition of peroxidase at 25°C. The increase of absorbance at 590 nm was monitored. The experiment was repeated twice with similar results, and each experiment had three replications. Data presented are from one experiment only.

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LITERATURE CITED

- Able, A. J., Guest, D. I., and Sutherland, M. W. 2000. Hydrogen peroxide yields during the incompatible interaction of tobacco suspension cells inoculated with *Phytophthora nicotianae*. *Plant Physiol.* 124:899-910.
- Adam, A., Farkas, T., Somlyai, G., Hevesi, M., and Kiraly, Z. 1989. Consequence of O₂⁻ generation during a bacterially induced hypersensitive reaction in tobacco: deterioration of membrane lipids. *Physiol. Mol. Plant Pathol.* 34:13-26.
- Altschul, J. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Baker, C. J., and Orlandi, E. W. 1995. Active oxygen in plant pathogenesis. *Annu. Rev. Phytopathol.* 33:299-321.
- Baker, C. J., O'Neill, N. R., Keppler, L. D., and Orlandi, E. W. 1991. Early response during plant-bacteria interactions in tobacco cell suspensions. *Phytopathology* 81:1504-1507.
- Breda, C., Sallaud, C., El-Turk, J., Buffard, D., De Kozak, I., Esnault, R., and Kondorosi, A. 1996. Defense reaction in *Medicago sativa*: A gene encoding a class 10 PR protein is expressed in vascular bundle. *Mol. Plant Microbe Interact.* 9:713-719.
- Büchter, R., Strömberg, A., Schemelzer, E., and Kombrink, E. 1997. Primary structure and expression of acidic (class III) chitinase in potato. *Plant Mol. Biol.* 35:749-761.
- Buffard, D., Breda, C., Van Huystee, R. B., Asemota, O., Pierre, M., Ha, D. B., and Esnault, R. 1990. Molecular cloning of complementary DNAs encoding two cationic peroxidase from cultivated peanut cells. *Proc. Natl. Acad. Sci. U.S.A.* 87:8874-8878.
- Chen, Z., Silva, H., and Klessig, D. F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* 262:1883-1886.
- Choi, Y. O., Cheong, N. E., Lee, K. O., Jung, B. G., Hong, C. H., Jeong, J. H., Chi, Y. H., Kim, K., Cho, M. J., and Lee, S. Y. 1999. Cloning and expression of a new isotype of the preoxiredoxin gene of Chinese cabbage and its comparison to 2Cys-peroxiredoxin isolated from the same plant. *Biochem. Biophys. Res. Commun.* 258:768-771.
- Chomczynski P., and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159.
- Dietrich, R. A., Richberg, M. H., Schmidt, R., Dean, C., and Dangle, J. L. 1997. A novel zinc finger protein is encoded by the Arabidopsis LSD1 gene and junctions a negative regulator of plant cell death. *Cell* 88:685-694.
- Doke, N. 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal cell wall components. *Physiol. Plant Pathol.* 23:359-367.
- Durner, J., and Klessig, D. F. 1995. Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense response. *Proc. Natl. Acad. Sci. U.S.A.* 92:11312-11316.
- Durner, J., and Klessig, D. F. 1996. Salicylic acid is a modulator of tobacco and mammalian catalases. *J. Biol. Chem.* 271:28492-28501.
- el-Turk, J., Asemota, O., Kondorosi, A., Leymarie, J., Sallaud, C., Mesnage, S., Breda, C., Buffard, D., Kondorosi, A., and Esnault, R. 1996. Nucleotide sequences of four pathogen-induced alfalfa peroxidase-encoding cDNAs. *Gene* 170:213-216.
- Eyal, Y., Meller, Y., Lev-Yadun, S., and Fluhr, R. 1993. A basic type PR-1 promoter directs ethylene responsiveness, vascular and abscission zone-specific expression. *Plant J.* 4:225-234.
- Fishel, L. A., Farnum, M. F., Mauro, J. M., Miller, M. A., and Kraut, J. 1991. Compound I radical in site-directed mutants of cytochrome c peroxidase as probed by electron paramagnetic resonance and electron-nuclear double resonance. *Biochemistry* 30:1986-1996.
- Gadea, J., Mayda, M. E., Conejero, V., and Vera, P. 1996. Characterization of defense-related genes ectopically expressed in viroid-infected tomato plants. *Mol. Plant-Microbe Interact.* 9:409-415.
- Gadea, J., Conejero, V., and Vera, P. 1999. Developmental regulation of a cytosolic ascorbate peroxidase gene from tomato plants. *Mol. Gen. Genet.* 262:212-219.
- Grant, J. J., and Loake, G. J. 2000. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. *Plant Physiol.* 124:21-29.
- Greenblatt, H. M., Ryan, C. A., and James, M. N. G. 1989. Structure of the complex of *Streptomyces griseus* proteinase b and polypeptide chymotrypsin inhibitor I from Russet Burbank potato tubers at 2.1 Å resolution. *J. Mol. Biol.* 205:201-228.
- Hammerschmidt, R., Nuckles, E., M., and Kuć, F. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.* 20:73-82.
- Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., and Matsui, H. 2001. A large family of Class III plant peroxidases. *Plant Cell Physiol.* 42:462-468.
- 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.
- Hückelhoven, R., Dechert, C., Trujillo, M., and Kogel, K. H. 2001. Differential expression of putative cell death regulator genes in near-isogenic, resistant and susceptible barley lines during interaction with the powdery mildew fungus. *Plant Mol. Biol.* 47:739-748.
- 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.
- Karpinski, S., Escobar, C., Karpinska, B., Creissen, G., and Mullineaux, P. M. 1997. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* 9:627-640.
- Keppler, L. D., and Baker, C. J. 1989. O₂-initiated lipid peroxidation in a bacteria-induced hypersensitive reaction in tobacco cell suspension. *Phytopathology* 79:555-562.
- Keppler, L. D., Baker, C. J., and Atkinson, M. M. 1989. Active oxygen production during a bacteria-induced hypersensitive reaction in tobacco suspension cells. *Phytopathology* 79:974-979.
- 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.
- Kubo, A., Saji, H., Tanaka, K., and Kondo, N. 1995. Expression of Arabidopsis cytosolic ascorbate peroxidase in response to ozone or sulfur dioxide. *Plant Mol. Biol.* 29:479-489.
- Lamb, C., and Dixon, R. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:251-275.
- Lee, S. C., Lee, Y. K., Kim, K. D., and Hwang, B. K. 2000a. In situ hybridization study of pathogen- and organ-dependent expression of a novel thionin gene in pepper (*Capsicum annuum*). *Physiol. Plant.* 110:384-392.
- Lee, Y. K., Hippe-Sanwald, S., Jung, H. W., Hong, J. K., Hause, B., and Hwang, B. K. 2000b. *In situ* localization of chitinase mRNA and protein in compatible and incompatible interactions of pepper stems with

- Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 57:111-121.
- 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.
- Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994. H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 70:583-593.
- Martinez, C., Baccou, J. C., Bresson, E., Baissac, Y., Daniel, J. F., Jalloul, A., Montillet, J. L., Geiger, J. P., Assigbetse, K., and Nicole, M. 2000. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of *Xanthomonas campestris* pv. *malvacearum*. *Plant Physiol.* 122:757-66.
- Mehdy, M. C. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiol.* 105:467-472.
- Mehdy, M. C., Sharma, Y. K., Sathasivan, K., and Bays, N. W. 1996. The role of activated oxygen species in plant disease resistance. *Physiol. Plant.* 98:365-374.
- Mittler, R., and Zilinskas, B. A. 1992. Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. *J. Biol. Chem.* 267:21802-21807.
- Mittler, R., Feng, X., and Chen, M. 1998. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *Plant Cell* 10:461-474.
- Morita, S., Kaminaka, H., Masumura, T., and Tanaka, K. 1999. Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress: The involvement of hydrogen peroxide in oxidative stress signaling. *Plant Cell Physiol.* 40:417-422.
- Muthukrishnan, S. 1999. Pages 171-193 in: *Pathogenesis-Related Proteins in Plants*. S. K. Datta, ed. CRC Press, New York.
- Okuda, T., Matsuda, Y., Yamanaka, A., and Sagisaka, S. 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. *Plant Physiol.* 97:1265-1267.
- Orvar, B. L., and Ellis, B. E. 1995. Isolation of a cDNA encoding cytosolic ascorbate peroxidase in tobacco. *Plant Physiol.* 108:839-840.
- Ostergaard, L., Teilum, K., Mirza, O., Mattsson, O., Petersen, M., Welinder, K. G., Mundy, J., Gajhede, M., and Henriksen, A. 2000. Arabidopsis ATP A2 peroxidase. Expression and high-resolution structure of a plant peroxidase with implications for lignification. *Plant Mol. Biol.* 44:231-243.
- Poulos, T. L., and Kraut, J. 1987. The stereochemistry of peroxidase catalysis. *J. Biol. Chem.* 255:8199-8205.
- Rao, M. V., Paliyath, G., Ormrod, D. P., Murr, D. P., and Watkins, C. B. 1997. Influence of salicylic acid on H₂O₂ production, oxidative stress and H₂O₂-metabolizing enzymes. *Plant Physiol.* 151:137-149.
- Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molin, A., Steiner, H., and Hunt, M. D. 1996. Systemic acquired resistance. *Plant Cell* 8:1809-1819.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Extraction, purification, and analysis of messenger RNA from eukaryotic cells. Pages 7.35-7.87 in: *Molecular Cloning: A Laboratory Manual*. N. Ford, C. Nolan, and M. Ferguson, eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
- Schantz, M. L., Schreiber, H., Guillemot, P., and Schantz, R. 1995. Changes in ascorbate peroxidase activities during fruit ripening in *Capsicum annuum*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 358:149-152.
- Vera-Estrella, R., Blumwald, E., and Higgins, V. J. 1992. Effect of specific elicitors of *Cladosporium fulvum* on tomato suspension cells. *Plant Physiol.* 87:206-210.
- Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Mtraux, J. P., and Ryals, J. A. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3:1085-1094.
- Welinder, K. G. 1985. Plant peroxidases. Their primary, secondary, tertiary structures, and relation to cytochrome peroxidase. *Eur. J. Biochem.* 151:497-504.
- Welinder, K. G. 1992. Superfamily of plant, fungal and bacterial peroxidase. *Curr. Opin. Struct. Biol.* 2:388-393.
- Yi, S. Y., and Hwang, B. K. 1998. Molecular cloning and characterization of a new basic peroxidase cDNA from soybean hypocotyls infected with *Phytophthora sojae* f. sp. *glycines*. *Mol. Cells* 8:556-564.
- Zhou, Y., Kok, K. H., Chun, A. C. S., Wong, C. M., Wu, H. W., Lin, M. C. M., Fung, P. C. W., Kung, H. F., and Jin, D. Y. 2000. Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. *Biochem. Biophys. Res. Commun.* 268:921-927.