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REVIEW

Molecular and cellular control of cell death and defense signaling in pepper

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Abstract Pepper (*Capsicum annuum* L.) provides a good experimental system for studying the molecular and functional genomics underlying the ability of plants to defend themselves against microbial pathogens. Cell death is a genetically programmed response that requires specific host cellular factors. Hypersensitive response (HR) is defined as rapid cell death in response to a pathogen attack. Pepper plants respond to pathogen attacks by activating genetically controlled HR- or disease-associated cell death. HR cell death, specifically in incompatible interactions between pepper and *Xanthomonas campestris* pv. *vesicatoria*, is mediated by the molecular genetics and biochemical machinery that underlie pathogen-induced cell death in plants. Gene expression profiles during the HR-like cell death response, virus-induced gene silencing and transient and transgenic overexpression approaches are used to isolate and identify HR- or disease-associated cell death genes in pepper plants. Reactive oxygen species, nitric oxide, cytosolic calcium ion and defense-related hormones such as salicylic acid, jasmonic acid, ethylene and abscisic acid are involved in the execution of pathogen-induced cell death in plants. In this review, we summarize recent molecular and cellular studies of the pepper cell death-mediated defense response, highlighting the signaling events of cell death in disease-resistant pepper plants. Comprehensive knowledge and understanding of the cellular functions of pepper cell death response genes will aid the development of novel practical approaches to enhance disease resistance in pepper, thereby helping to secure the future supply of safe and nutritious pepper plants worldwide.

Keywords *Capsicum annuum* · Hypersensitive cell death · Defense · Cell death gene · Disease resistance · Disease-associated cell death

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Introduction

Pepper (*Capsicum* spp.), a member of the Solanaceae family, is extensively cultivated worldwide for nutritional and condimental use. According to a report from the United States Department of Agriculture, world pepper production almost tripled from about 10 million tons to over 26 million tons between 1990 and 2007 (http://usda.mannlib.cornell.edu). Pepper fruit is consumed as a fresh vegetable or dehydrated for use as a spice. By volume, red pepper products, pungent and nonpungent, are one of the most important spice commodities in the world. Peppers add flavor and color to foods while providing essential vitamins and minerals (Pernezny et al. 2003). Several pathogens, such as *Phytophthora* spp., *Colletotrichum* spp. and *Xanthomonas* spp., cause excessive losses in pepper production (Hwang and Kim 1995; Stall et al. 2009). The current measures used to prevent the devastating diseases of pepper plants include the application of chemicals, cultural practices and the use of disease-resistant cultivars (Hwang and Kim 1995; Sahin and Miller 1998; Choi and Hwang 2011b). Among them, the cultivation of disease-

resistant cultivars is one of the most cost-effective and environmentally friendly disease control methods.

Hypersensitive response (HR) cell death is one of the most effective responses of pepper plants to attacks by various pathogens. This response confines the pathogen to the initial infection site, thereby restricting the growth and spread of the pathogen (Stall et al. 2009). A specific HR cell death phenotype is observed in pepper leaves that are inoculated with the avirulent *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), but not in those that are inoculated with virulent *Xcv* (Lee and Hwang 2005; Choi et al. 2007). Therefore, a comprehensive understanding of the molecular and biochemical basis of HR cell death in pepper will provide new insights into how pepper plants can appropriately respond to a wide array of microbial pathogens.

Programmed cell death, an intrinsic program of cell suicide, occurs in many plant–pathogen interactions and is frequently associated with the activation of plant innate immunity (Gadjev et al. 2008). The most well-known form of plant programmed cell death is HR, which is activated by intracellular resistance (*R*) protein-mediated recognition of effector proteins from avirulent pathogens (Chisholm et al. 2006; Jones and Dangl 2006). This phenomenon is referred to as effector-triggered immunity (ETI; reviewed by Jones and Dangl 2006; Stall et al. 2009). Despite the identification of some effector proteins from the pepper bacterial spot pathogen *Xcv* (Escolar et al. 2001; Büttner et al. 2003; Kim et al. 2010; Schulze et al. 2012), their cognate *R*-genes remain to be identified. Two nucleotide-binding site leucine-rich repeat (NBS-LRR) classes of *R*-genes, namely, *Bs2* and *CaMi,* were isolated and functionally characterized from pepper plants resistant to *Xcv* and a root-knot nematode (*Meloidogyne* spp.), respectively (Tai et al. 1999; Chen et al. 2007). Wan et al. (2012) recently identified 78 putative pepper *R*-gene analogs (*CaRGAs*) by degenerate PCR amplification and database mining. Among them, 51 *CaRGAs* showed homology with NBS-LRR class R proteins from Arabidopsis and tomato. Notably, *CaRGA2* expression confers resistance of pepper plants to *Phytophthora capsici* (Zhang et al. 2013). Further functional studies of the *CaRGAs* are required to understand the molecular and cellular mechanisms underlying the *R*-gene- and cell death-mediated resistance response of pepper plants.

Studies of the *Xcv* effector protein AvrBs3 and its cognate *R*-gene loci in the pepper ECW-30R background revealed that a novel type of *R*-gene, namely, *Bs3*, shares homology with Arabidopsis flavin-dependent monooxygenase 1 (FMO1) (van den Ackerveken et al. 1996; Römer et al. 2007). The *Xcv* effector protein AvrBs3 is neither directly nor indirectly recognized by the Bs3 protein, but binds and activates the promoter region of *Bs3*. AvrBs3-dependent or 35S-driven expression of *Bs3* triggers HR cell death. Notably, AvrBs3 also changes the transcriptome of susceptible pepper plants to the benefit of the pathogen (Kay et al. 2007; Strauss et al. 2012), leading to effector-triggered susceptibility (ETS, reviewed by Jones and Dangl 2006). Further studies of the AvrBs3 effector protein family have defined nuclear localization sequences, specific DNA-binding domains and acidic activation domains, and these proteins are thus named transcription activator-like effectors (reviewed by Boch and Bonas 2010).

Upon avirulent pathogen infection, pepper plants activate dynamic and multilevel defense responses during HR cell death including oxidative burst, calcium ion (Ca^{2+}) influx, salicylic acid (SA) accumulation and transcriptional activation of pathogenesis-related (*PR*) genes, ultimately leading to the restriction of pathogen growth (Greenberg and Yao 2004; Lee and Hwang 2005; Jones and Dangl 2006). In pepper, a variety of HR cell death response genes have been identified and functionally characterized to define their roles in HR cell death-mediated defense signaling. Molecular, cellular and biochemical analyses have revealed parts of the complex signal transduction pathways that control pathogen-induced hypersensitive cell death events in pepper. In this review, we summarize the molecular and cellular signaling mechanisms underlying HR cell death gene-mediated disease resistance and susceptibility responses that have been studied in pepper to date.

Molecular and cellular basis of HR cell death and defense responses in pepper

In an effort to understand the molecular and cellular basis of cell death and defense responses of pepper plants to various pathogens, HR cell death-associated genes have been isolated and extensively identified during the past decades. In particular, the pepper–*Xcv* pathosystem has been used to identify pepper HR cell death and defense response genes encoding proteins that (1) mediate specific recognition and downstream signaling, (2) are involved in intracellular calcium signaling, (3) modulate the levels of key signaling intermediates such as reactive oxygen species (ROS) and SA, (4) regulate gene transcription, (5) alter metabolic pathways, (6) regulate lipid-associated defense signaling, (7) trigger cell wall-based defense, (8) act in antimicrobial defense, (9) regulate the cell death response and (10) are involved in pathogenesis.

The transcriptome and proteome of pepper leaves infected with *Xcv*

Recent technical advances and the development of bioinformatics approaches have allowed us to monitor global

changes in gene expression and protein levels in plants. Molecular and biochemical changes that occur in plants exposed to pathogens can be identified through high-throughput transcriptome and proteome analyses (Maleck et al. 2004; Jones et al. 2006). Differential hybridization technique has been successfully used to isolate defense-related genes ectopically expressed in *Xcv*-infected pepper plants (Jung and Hwang 2000a, b; Choi et al. 2007; Hwang et al. 2014a). It seems to be an effective strategy for the isolation of a large number of cell death and defense response genes in pepper plants. Specific cDNAs showing differential expression in *Xcv*-infected pepper leaves as opposed to healthy leaves were isolated from a pepper cDNA library from HR lesions of leaves infected with the *Xcv* avirulent strain Bv5-4a (Jung and Hwang 2000a, b). Among a total of 282 cDNA clones tested, 36 individual cDNA genes (13 %) hybridized strongly or differentially to the cDNA probes from *Xcv*-infected leaves. Transcripts of the *Capsicum annuum*-induced (*CAI*) genes encoding putative thionin, lipid transfer protein I and II, osmotin (PR-5), class I chitinase, β-1,3-glucanase, SAR 8.2, stellacyanin, leucine-rich repeat (LRR) protein and auxin-repressed protein were strongly or preferentially induced in pepper tissues by infection with *Xcv* or *P. capsici* and by abiotic elicitor treatment (Jung and Hwang 2000a, b). Based on the data of research articles published by Hwang's research group over the past decade, transcriptional expression patterns of some HR cell death- or defense-related genes in pepper plants are shown in Fig. 1. Spatial and temporal expression of the pepper genes has been recognized as inducible defense response in pepper plants. No transcripts of all the pepper defense response genes tested were detected in pepper leaves (Fig. 1a), indicating that these genes are not constitutively expressed in leaves. Notably, *CaMBL1* and *CaMRP1* were not transcriptionally expressed in all the healthy pepper tissues. However, their constitutive expression in the other pepper organs varied according to the different defense response genes. Figure 1b shows the transcription patterns of the pepper defense response genes in pepper leaves infected with *Xcv* virulent (compatible) strain Ds1 and avirulent (incompatible) strain Bv5-4a. Overall, the HR cell death- or defense-related genes were significantly and strongly induced in pepper leaves at early time points after

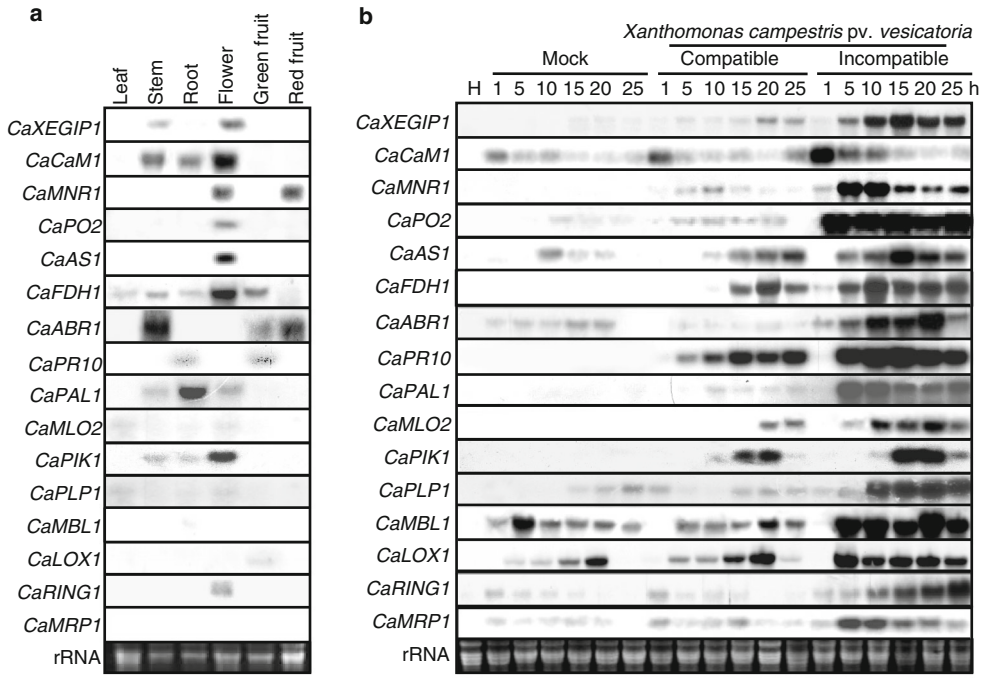

Fig. 1 Transcriptional expression of HR cell death- or defense-related genes in pepper plants. **a** Expression in heathy pepper organs. **b** Expression patterns in pepper leaves infected with *X. campestris* pv. *vesicatoria* virulent (compatible) strain Ds1 and avirulent (incompatible) strain Bv5-4a. *Mock* treated with 10 mM $MgCl_2$, *H* healthy leaves, *CaXEGIP1* xyloglucan-specific endo-β-1,4-glucanase inhibitor (Choi et al. 2013c), *CaCaM1* calmodulin (Choi et al. 2009), *CaMNR1* menthone reductase (Choi et al. 2008), *CaPO2* (Choi et al. 2007), *CaAS1* asparagine synthetase (Hwang et al. 2011), *CaFDH1* formate dehydrogenase (Choi et al. 2014, *CaABR1* ABA-responsive protein (Choi and Hwang 2011a), *CaPR10* pathogenesis-related protein (Choi et al. 2012), *CaPAL1* phenylalanine ammonia-lyase (Kim and Hwang 2014), *CaMLO2* mildew resistance locus O (Kim and Hwang 2012), *CaPIK1* receptor-like cytoplasmic kinase (Kim and Hwang 2011), *CaPLP1* patatin-like phospholipase (Kim et al. 2014a), *CaMBL1* mannose-binding lectin (Hwang and Hwang 2011), *CaLOX1* 9-lipoxygenase (Hwang and Hwang 2010), *CaRING1* E3 ubiquitin ligase RING1 (Lee et al. 2011a), *CaMRP1* membrane-located receptor-like protein (An et al. 2008a)

inoculation with the avirulent *Xcv* Bv5-4a, compared to their induction by the mock or virulent *Xcv* Ds1 inoculations. Together, these data support the notion that the avirulent *Xcv* Bv5-4a infection triggers the induction of HR cell death- or defense-related genes in pepper plants. However, the transcriptional changes may not reflect regulatory processes at the whole cell level, since there are many different post-transcriptional and post-translational modifications in pepper plants.

Proteomics, a powerful tool for dissecting complex protein mixtures, has been employed to analyze plant proteins induced by both biotic and abiotic stresses (Zang and Komatsu 2006). In contrast to transcriptional activation profiling, proteomics analysis monitors the actual protein composition of cells in specific tissues, which is directly influenced by the biochemical cellular pathway (Colditz et al. 2005). The proteome of pepper leaves inoculated with the *Xcv* virulent strain Ds1 or the avirulent strain Bv5-4a was analyzed to identify pepper cell death- or defense-related proteins using two-dimensional (2D) gel electrophoresis, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry and liquid chromatography–tandem mass spectrometry (LC/MS–MS) (Choi and Hwang 2011a, b). The Solanaceae EST Analysis System (http://sol.pdrc.re.kr/) was used to functionally categorize pepper proteins that were identified. *Arabidopsis* orthologs of the identified pepper proteins were also used to functionally categorize the pepper proteins into six classes based on the MIPS *Arabidopsis thaliana* genome database (MatDB, http://mips.gsf.de/proj/funcatDB/search_main_frame.html): C-compound and carbohydrate metabolism (5.5 %), photosynthesis (5.5 %), RNA synthesis (5.5 %), extracellular/secretion (5.5 %), disease, virulence and defense (61 %) and unclassified proteins (17 %) (Choi and Hwang 2011a). Most of the novel proteins, induced in pepper leaves during *Xcv* infection, were associated with disease, virulence and defense. Among the defense-related proteins identified was CaABR1, a GRAM domain-containing ABA-responsive protein. *CaABR1* was highly induced by infection with avirulent *Xcv* and also by treatment with ABA. To identify proteins whose expression was altered by *CaHIR1* (hypersensitive induced reaction protein) overexpression in *Arabidopsis* leaves, a quantitative comparative proteome analysis using 2-D gel electrophoresis coupled with mass spectrometry was performed (Jung et al. 2008). Of about 400 soluble proteins, 11 proteins such as glycine decarboxylase, carbonic anhydrase and superoxide dismutase that are involved in several metabolic pathways were up- or down-regulated by *CaHIR1* overexpression in transgenic *Arabidopsis* leaves.

Differential display and micro- or macroarrays of the transcriptome and proteome in pepper allow for quantitative comparisons of protein expression levels between healthy and diseased pepper plants. A combination of genetic and proteomic approaches reveals incompatible interactions that regulate the resistance response of pepper plants to pathogens. Transcriptomics and proteomics analyses of *Xcv*–pepper interactions will lead to further definition of the role of defense signaling-related proteins in cell death and defense responses against microbial pathogens.

HR cell death and defense genes

Gene expression profiles during the HR-like cell death response to avirulent microbial pathogens, virus-induced gene silencing (VIGS) and transient and transgenic overexpression approaches are used to isolate and identify HR cell death and defense response genes in pepper plants. Notably, most cell death and defense response genes were isolated from HR leaves of pepper plants infected with avirulent *Xcv* using a differential hybridization technique (Jung and Hwang 2000a). Genetic, biochemical and cell biological studies of pepper HR revealed that activation of HR cell death and defense responses is linked to signal transduction pathways, which are coordinated by the actions of defense signaling molecules, such as ROS [superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH)], nitric oxide (NO), calcium ions (Ca^{2+}) and the plant defense hormones.

Receptor-like proteins

The plant cell membrane encloses the cell contents and separates them from the surrounding environment. Plasma membrane-localized receptor-like proteins and genes, such as receptor-like kinase 1 (*CaRLK1*) (Yi et al. 2010), *Pathogen-Induced Membrane Protein 1* (*CaPIMP1*) (Hong et al. 2008a), *Membrane-Located Receptor-Like Protein 1* (*CaMRP1*) (An et al. 2008a), *Mannose-Binding Lectin* (*CaMBL1*) (Hwang and Hwang 2011) and *Mildew Resistance Locus O* (*CaMLO1* and *CaMLO2*) (Panstruga 2005; Kim and Hwang 2012; Zheng et al. 2013), are distinctly expressed during HR cell death to regulate the plant defense response (Table 1). Transgenic expression of *CaRLK1* significantly compromised virulent and avirulent bacterial pathogen-induced cell death in *Nicotiana benthamiana,* accompanied by production of the superoxide anion and induction of a negative regulator of the pro-death pathway component *Lesions Simulating Disease* (*LSD*) gene (Yi et al. 2010). This suggests that *CaRLK1* negatively regulates HR cell death in plants. By contrast, the *CaPIMP1* and *CaMBL1* genes, which encode transmembrane proteins, positively regulated HR cell death and the HR cell death-mediated defense response of transgenic Arabidopsis plants against *Pseudomonas syringae* pv.

Table 1 Functional classification of cell death and defense response genes in pepper

Gene name	Accession no.	Putative function	Localization	Inducer	References
Receptor-like protein					
CaRLK1	EF397556	Receptor-like kinase	PM (PM)	*Xcv*, *Xag* and H_2O_2	Yi et al. (2010)
CaPIMP1	DQ356278	Membrane protein	PM (Ch, PM)	*Xcv*	Hong et al. (2008a)
CaMRP1	EU181138	Membrane receptor-like protein	PM (PM)	*Xcv*, *C. coccodes*, JA and wound	An et al. (2008a)
CaMLO1	AAX31277	Mildew resistance locus O	N.D. (PM)		Panstruga (2005)
CaMLO2	JN896629	Mildew resistance locus O	PM (PM)	*Xcv*, *P. capsici*, SA, JA and Mv	Kim and Hwang (2012)
CaMBL1	GQ265892	Mannose-binding lectin	PM (PM)	*Xcv* and SA	Hwang and Hwang (2011)
Protein kinase					
CaPIK1	GU295436	Receptor-like cytoplasmic Kinase	C, PM (C)	*Xcv*, SA, JA ET and Mv	Kim and Hwang (2011)
CaPKc1	DQ114474	Pyruvate kinase	C (PM, C)	TMV, SA, JA and ET	Kim et al. (2006)
CaMK1	AF247135	MAP kinase	N.D. (C)	Wound	Shin et al. (2001)
CaMK2	AF247136	MAP kinase	N.D. (Ch)		Shin et al. (2001)
Calcium sensor protein					
CaCaM1	EU935058	Calmodulin	N.D. (C)	*Xcv*, H_2O_2, SA, JA, ABA and wound	Choi et al. (2009)
CaCDPK3	AY295081	Calcium-dependent protein kinase	C(C)	*Xag*, SA, ET, JA and ABA	Chung et al. (2004)
CaCDPK4	AY904339	Calcium-dependent protein kinase	N.D. (PM, N)	*Xag*, SA, ET, JA, and ABA	Chung et al. (2007)
Redox-associated protein					
CaPO1	AF442386	Peroxidase	N.D. (PM)	*Xcv*, *P. capsici* and H_2O_2	Do et al. (2003)
CaPO2	DQ489711	Peroxidase	EM (EM)	*Xcv* and ABA	Choi et al. (2007)
CaPOA1	AF442387	Ascorbate peroxidase	EM (PM)	*Xcv*, *P. capsici*, H_2O_2 and SA	Do et al. (2003)
CaPOT1	AF442385	Thioredoxin peroxidase	N.D. (C)	*Xcv*, *P. capsici*, H_2O_2 and SA	Do et al. (2003)
CaCAT1	AF227952	Catalase	N.D. (P)	Mv	Lee and An (2005)
CaCAT2	AY128694	Catalase	N.D. (C)	Mv	Lee and An (2005)
CaCAT3	AY128695	Catalase	N.D. (P)	Mv	Lee and An (2005)
CaMsrB2	EF144171	Methionine sulfoxide reductase	C (ER)		Oh et al. (2010)
Transcription factor					
CaZFP1	AF539746	Cys2/His2-type zinc-finger transcription factor (TF)	N (C)	*Xcv*, *C. coccodes*, SA, JA, ET, ABA, H_2O_2 and wound	Kim et al. (2004)
CaRAV1	AF478458	RAV (Related to ABI3/VP1) TF	N (N)	*Xcv*, SA, JA, ET, ABA, H_2O_2 and wound	Sohn et al. (2006)
CabZIP1	AY775332	Basic leucine zipper (bZIP) TF	N (N, ER)	*Xcv*, SA, JA, ET, ABA, H_2O_2 and Mv	Lee et al. (2006)
CaWRKY1	EF468464	WRKY TF	N.D. (C)	*Xcv*, *Xag*, PMMOV and SA	Oh et al. (2008)
CaWRKY2	DQ402241	WRKY TF	N (PM, G)	*Xcv*, *Xag*, PMMOV, SA, JA, ET and wound	Oh et al. (2006)
CaWRKY-a	AY391747	WRKY TF	N (Ch)	*Xcv*, TMV, SA, JA, ET and wound	Park et al. (2006)
CaATL1	DQ472735	AT-hook motif-containing TF	N (N)	*Xcv*, *Xag*, TMV, SA and ET	Kim et al. (2007b)
CaPF1	AY246274	ERF/AP2-type TF	N.D. (N)	*Xcv*, *Xag*, JA and ET	Yi et al. (2004)
CaNAC1	AY714222	NAC family TF	N (P)	*Xag*, *Xcv*, *P. syringae*, SA, ET, JA and wound	Oh et al. (2005)

Table 1 continued

Gene name	Accession no.	Putative function	Localization	Inducer	References
CaERFLP1	AY529642	ERF/AP2-type TF	N (N)	*Xag* and ET	Lee et al. (2004)
CaCAF1	DQ672569	CCR4-associated factor	N.D. (P)	N.D.	Sarowar et al. (2007)
Secondary metabolite-associated protein					
CaMNR1	EF576664	Menthone reductase	N.D. (C)	*Xcv*, SA, ET, and ABA	Choi et al. (2008)
CaADC1	KC160547	Arginine decarboxylase	C (C)	*Xcv*, SA, JA, and ETH	Kim et al. (2013a)
CaPAL1	KF279696	Phenylalanine ammonia-lyase	N.D. (PM, Ch)	*Xcv*, SA	Kim and Hwang (2014)
Lipid metabolism-associated protein					
CaGL1	DQ119290	Lipase	EM (EM)	TMV, JA and wound	Kim et al. (2008)
CaGLIP1	AY775336	Lipase	EM (ER)	*Xcv*, SA, JA, ET, Mv and wound	Hong et al. (2008b)
CaFAD1	EF645679	Fatty acid desaturase	N.D. (PM)	TMV and *Xcv*	Kim et al. (2007a)
CaLOX1	FJ377808	9-Lipoxygenase	N.D. (P)	*Xcv*, SA, JA, ETand Mv	Hwang and Hwang (2010)
CaLTP1	AF208832	Lipid transfer protein	N.D. (C)	*Xcv*, *P. capsici*, SA, JA, ET and ABA	Jung et al. (2003), Sarowar et al. (2009)
CaLTP2	AF208833	Lipid transfer protein	N.D. (V, EM)	*Xcv*, *P. capsici* and JA	Jung et al. (2003), Sarowar et al. (2009)
CaLTP3	AF208834	Lipid transfer protein	N.D. (EM)	*Xcv*, *P. capsici*, JA, ET, ABA and wound	Jung et al. (2003)
CaPLP1	HM231272	Patatin-like phospholipase	C, PM (PM, ER)	*Xcv* and SA	Kim et al. (2014a)
Cell wall protein					
CaXEGIP1	JQ673414	Xyloglucan-specific endo-β-1,4-glucanase inhibitor	EM (EM)	*Xcv*	Choi et al. (2013a, b)
CaPRP1	JF904891	Proline-rich protein	N.D. (PM)		Yeom et al. (2012)
Antimicrobial protein					
CaAMP1	AY548741	Antimicrobial protein	EM (C)	*Xcv*, *P. capsici*, SA, JA, ET, H_2O_2 and wound	Lee et al. (2008, 2011b)
CaSAR82	AF112868	Antimicrobial protein	N.D. (EM, V)	*Xcv*, *P. capsici*, SA, JA, ET, H_2O_2 and ABA	Lee and Hwang (2003)
CaCBP1	AF112867	Chitin-binding protein	N.D. (EM)	*Xcv*, *C. coccodes*, *P. capsici*, JA, ET and wound	Lee et al. (2001)
CaPMEI1	AF477956	Pectin methylesterase inhibitor	N.D. (C)	*Xcv*, *C. coccodes*, SA, JA, ET, ABA, H_2O_2 and wound	An et al. (2008b)
Cell death-associated protein					
CaLRR1	AY237117	Leucine-rich repeat protein	EM (EM)	*Xcv*, *C. coccodes*, *P. capsici*, ABA and wound	Jung et al. (2004), Jung and Hwang (2007), Choi et al. (2012)
CaHIR1	AY529867	SPFH domain-containing protein	PM (C)	*Xcv*, SA, JA, ET and ABA	Jung and Hwang (2007), Jung et al. (2008), Choi et al. (2011)
CaBI-1	FJ719768	Bax inhibitor	N.D. (PM)	ABA, high temperature and heavy metals	Ishikawa et al. (2011)
CaABR1	GQ373000	ABA-responsive protein	N (Mb, C)	*Xcv*	Choi and Hwang (2011a)
Camc9	KC597255	Metacaspase	N.D. (ER)	*Xcv* and JA	Kim et al. (2013b)

Table 1 continued

Gene name	Accession no.	Putative function	Localization	Inducer	References
CaRBP1	DQ864657	RNA-binding protein	N(N)	*Xcv*	Lee et al. (2012)
CaRING1	GQ359822	E3 ubiquitin ligase	PM (ER, Ch)	*Xcv*	Lee et al. (2011a)
CaDC1	HM581976	DC1 domain protein	N, C (C, Mb)	*Xcv*	Hwang et al. (2014a)
Pathogenesis-related protein					
CaPR1	AF053343	Pathogenesis-related 1 (PR 1)	N.D. (EM)	*Xcv,* SA, ET and JA	Kim and Hwang (2000), Sarowar et al. (2005)
CaPR4b	HM581975	PR 4	ER, PM (ER)	*Xcv*	Hwang et al. (2014b)
CaPR10	AF244121	Ribonuclease	N.D. (M, C)	*Xcv,* SA, ET and JA	Choi et al. (2012)
CaOSM1	AY262059	Osmotin-like protein	PM (EM)	*Xcv*, *C. coccodes*, *P. capsici*, ET and JA	Hong et al. (2004), Choi et al. (2013a)
CaChi2	AF091235	Chitinase	N.D. (EM)	*Xcv,* SA, ET and JA	Hong et al. (2000)
CaThi1	AF112869	Thionin	N.D. (EM)	*Xcv*	Lee et al. (2000)
CaBGLU1	AF082724	β-1,3-glucanase	N.D. (EM)	*Xcv,* ET and JA	Jung and Hwang (2000b)
CaCBP1	AF112867	Chitin-binding protein	N.D. (EM)	*Xcv,* ET and JA	Lee et al. (2001)
CaDEF1	AF442388	Defensin	N.D. (EM)	*Xcv*, SA, JA, ABA, H_2O_2 and wound	Do et al. (2004)

The abbreviations in parentheses indicate the predicted subcellular localizations of proteins based on the PSORT database (http://psort.ims.u-tokyo.ac.jp/form.html)

Xcv avirulent strains of *X. campestris* pv. *vesicatoria*, which cause a resistant response and elicit a hypersensitive response in pepper, *Xag* non-host pathogen *X. axonopodis* pv. *glycines*, which elicits hypersensitive cell death in pepper, *TMV* tobacco mosaic virus strain P_0, which causes a resistant response and elicits hypersensitive cell death in pepper, *PMMOV* pepper mild mottle virus, which causes a hypersensitive response in pepper

PM plasma membrane, *C* cytoplasm, *Ch* chloroplast, *EM* extracellular matrix, *N* nucleus, *P* peroxisome, *ER* endoplasmic reticulum, *G* Golgi body, *V* vacuole, *M* mitochondria, *Mb* microbody, *N.D.* not determined, *SA* salicylic acid, *JA* jasmonic acid, *ET* ethylene, *ABA* abscisic acid, *Mv* methyl viologen (paraquot)

tomato (*Pst*) infection (Hong et al. 2008a; Hwang and Hwang 2011). Notably, it is hypothesized that CaMBL1 recognizes pathogen-released extracellular matrix-containing glycoproteins with mannose (Man) residues, based on its in vitro D-Man-binding activity and in planta HR cell death-inducing activity. *CaMRP1*, a homolog of the tobacco osmosensor-like membrane protein NtC7 (Tamura et al. 2003), negatively regulates plant defense (An et al. 2008a). VIGS of *CaMRP1* reduced lesion formation and bacterial growth in virulent *Xcv*-inoculated pepper plants. The pepper *MLO* genes *CaMLO1* and *CaMLO2* negatively regulated defense responses in pepper plants (Kim and Hwang 2012; Zheng et al. 2013). Silencing of *CaMLO2* enhanced broad-spectrum resistance of pepper against bacterial spot (*Xcv*) and powdery mildew (*Leveillula taurica*) pathogens.

Essential proteins that have receptor domains on either side of the membrane control cell-signaling responses to a large variety of environmental stimuli (Breton et al. 2003). These membrane proteins can recognize and transport signals relating to environmental changes, such as pathogen infection, drought, high salinity and cold stress. In conclusion, some receptor-like proteins such as CaRLK1, CaMRP1 and CaMLO negatively regulate cell death and defense responses in pepper plants; however, other transmembrane proteins CaPIMP1 and CaMBL1 contribute positively to the HR cell death. These membrane-located membrane proteins may interact with different pathogen factors, as well as host molecules such as protein kinases and transcription factors to activate downstream disease- or defense–related responses. More recently, it has been demonstrated that pepper CaMLO2 specifically interacts with CaCaM1 and translocates cytoplasmic CaCaM1 to the plasma membrane, leading to the suppression of *Xanthomonas* AvrBsT-triggered hypersensitive cell death and defense responses (Kim et al. 2014c).

Protein kinases

Along with receptor-like proteins, mitogen-activated protein kinases and cytosolic kinases are intimately linked with the signal transduction of extracellular stimuli into cellular responses by regulating the phosphorylation status of a specific target protein (Meng and Zhang 2013). The mitogen-activated protein kinase-encoding genes *CaMK1* and *CaMK2* were isolated from pepper (Shin et al. 2001). CaMK1 and CaMK2 show a high level of amino acid homology to tobacco wound-induced protein kinase and SA-induced protein kinase, respectively. However, their roles in HR cell death and resistance responses are unknown. In pepper plants, cytoplasmic kinase genes, such as *Pyruvate Kinase 1* (*CaPKc1*) (Kim et al. 2006) and *Receptor-Like Cytoplasmic Protein Kinase 1* (*CaPIK1*) (Kim and Hwang 2011), are transcriptionally activated during HR cell death (Table 1). Pyruvate kinase is an important regulatory enzyme of the glycolytic pathway that catalyzes the transfer of Pi from phosphoenolpyruvate to ADP, thereby yielding pyruvate and ATP (Kim et al. 2006). Thus, pathogen-induced CaPKc1 is proposed to participate in ATP generation to provide the energy required for HR cell death and defense responses. CaPIK1 interacts with the pepper SGT1 (suppressor of the G2 allele of *skp1*) in yeast and in planta (Kim et al. 2014b). CaPIK1 showed in vitro autophosphorylation and myelin basic protein phosphorylation activities (Kim and Hwang 2011). More importantly, mutation of the predicted ATP-binding site (K130R) or kinase active site (D228H) of CaPIK1 led to the loss of its autophosphorylation and myelin basic protein phosphorylation activities, as well as the loss of its pathogen-independent cell death-inducing activity in pepper plants. This suggests that the cell death-inducing activity of CaPIK1 in plants is phosphorylation dependent.

Protein kinases are crucial for the recognition of pathogens and defense responses in plants (Romeis 2001; Afzal et al. 2008). Pepper pathogen-induced protein kinase *CaPIK1* plays a key role in signal transduction by phosphorylating target proteins involved in plant defense response (Kim and Hwang 2011). More recently, we proposed a working model for the *Xanthomonas* effector AvrBsT recognition of CaSGT1 to promote HR cell death associated with CaPIK1-mediated phosphorylation (Kim et al. 2014b).

Liquid chromatography–tandom mass spectrometry (LC/MS/MS) of the proteolytic peptides of CaSGT1 identified the residues Serine 98 and Serine 279 of CaSGT1 as the major CaPIK1-mediated phosphorylation sites. Together, these studies conclude that AvrBsT promotes HR cell death associated with CaPIK1-mediated phosphorylation by specifically interacting with CaSGT1 (Kim et al. 2014b).

Calcium sensor proteins

An increase in cytosolic-free calcium ($[Ca^{2+}]_{cyt}$) owing to the influx of Ca^{2+} is crucial to activate HR cell death and defense-related signal transduction (Lecourieux et al. 2006; Ma and Berkowitz 2007). The increase in $[Ca^{2+}]_{cyt}$ activates defense signaling components either by directly activating a group of Ca^{2+}-dependent protein kinases (CDPKs) (Roberts 1993; Romeis et al. 2001; Chung et al. 2004; Ludwig et al. 2004) or by indirectly activating target proteins through Ca^{2+}-binding proteins, such as calmodulin (CaM) and CaM-like proteins (Kim et al. 2002; Bouché et al. 2005; Popescu et al. 2007; Ma et al. 2008; Choi et al. 2009). In plants, Ca^{2+}-bound CaM activates NAD kinase to catalyze the conversion of NAD to NADP and the

resulting NADP is proposed to enhance the levels of NADPH, which functions as a reductant in an oxidative burst reaction (Harding et al. 1997).

Recently, the increase in $[Ca^{2+}]_{cyt}$ was demonstrated to activate pepper CaM (CaCaM1) to induce downstream NO and an oxidative burst, which ultimately led to HR-like cell death, *PR* gene expression and local acquired resistance (Choi et al. 2009) (Table 1). Expression of *CaCaM1* was strongly induced in pepper leaves during the HR upon avirulent *Xcv* inoculation. Treatment of pepper leaves with the calcium channel blocker $LaCl_3$ significantly suppressed *CaCaM1*-regulated radical bursts and subsequent HR-like cell death, suggesting that calcium influx-mediated activation of the CaCaM1 protein mediates cell death in pepper plants (Choi et al. 2009). In pepper, *CaCDPK3* and *CaCDPK4*, which are CDPK homologs, were isolated during biotic and abiotic stresses (Chung et al. 2004, 2007). However, the detailed function of these *CDPK* genes in pepper cell death or defense responses is not understood.

Calcium signaling has emerged as an important signal transduction pathway of higher plants in response to biotic and abiotic stresses. Ca^{2+}-bound calmodulin (CaM) plays a critical role in decoding and transducing stress signals by activating specific targets (Choi et al. 2009). CaCaM1 specifically interacts with CaMLO2, and cytosolic CaCaM1 gets translocated to the plasma membrane (PM) where CaMLO2 localizes (Kim et al. 2014c). CaCaM1 and CaMLO2 negatively regulated AvrBsT-triggered hypersensitive cell death in *N. benthamiana* leaves. Together, we conclude that *CaCaM1* expression alone induces HR cell death, but co-expression of *CaCaM1* with *CaMLO2* attenuates cell death and defense response.

Redox-associated proteins

Perturbation of intracellular redox regulation or activation of oxidative signaling pathways plays a key role in HR cell death (Gadjev et al. 2008; De Pinto et al. 2012). Most ROS-scavenging peroxidases and catalases negatively regulate HR cell death by protecting plant cells from oxidative burst (Gadjev et al. 2008). Pepper ascorbate peroxidase-like (*CAPOA1*), thioredoxin peroxidase-like (*CAPOT1*) and peroxidase-like (*CAPO1*) genes were down-regulated at the early time after *Xcv* infection when the HR was visible (Do et al. 2003). By contrast, a pH-dependent cell wall peroxidase was proposed to generate ROS upon pathogen-induced transient alkalization of the extracellular matrix (Zimmerlin et al. 1994). In French bean (*Phaseolus vulgaris*) suspension-cultured cells, a cell wall peroxidase is responsible for ROS production (Bolwell et al. 1998). Pepper cell wall peroxidase-dependent ROS generation induces pathogen-dependent HR-like cell death and enhances disease resistance in pepper and Arabidopsis plants (Choi et al. 2007). In pepper leaves, silencing of *CaPO2* compromised avirulent *Xcv*-induced H_2O_2 accumulation and HR cell death. Notably, avirulent *Xcv*-induced systemic microoxidative burst and micro-HR cell death phenotypes were abolished by treatment with the peroxidase inhibitor potassium cyanide (Choi et al. 2007). By contrast, transgenic overexpression of *CaPO2* conferred disease resistance accompanied by an HR cell death phenotype, as well as tolerance to multiple stresses in Arabidopsis (Choi et al. 2007; Choi and Hwang 2012). Treatment with diphenyleneiodonium chloride (an NADPH oxidase complex inhibitor) also compromised avirulent *Xcv*-induced systemic acquired resistance, systemic microoxidative bursts and micro-HR in pepper plants (Lee and Hwang 2005; Choi et al. 2007) (Table 1). This suggests that both extracellular peroxidase- and NADPH oxidase-dependent ROS generation is required for resistance responses, specifically for the cell death-mediated defense response in pepper. Pepper catalase genes (*CaCat*) are also suggested to play a significant role in response to environmental stresses such as methyl viologen (paraquat) and wounding (Lee and An 2005).

The ROS burst can damage DNA, lipids and proteins, especially those with the sulfur-containing amino acid methionine (Met) (Gao et al. 1998). To repair Met-oxidized proteins, Met sulfoxide reductase enzymes catalyze the reduction of Met sulfoxide into Met (Stadtman et al. 2005). In pepper plants, *CaMsrB2* was down-regulated upon inoculation with incompatible or compatible pathogens (Oh et al. 2010). Importantly, *CaMsrB2*-silenced pepper plants exhibited accelerated HR cell death upon avirulent *X. campestris* pv. *vesicatoria* infection. This suggests that CaMsrB2-induced Met reduction negatively regulates the HR cell death response in pepper plants.

In addition to an ROS burst, rapid production of NO has been implicated in plant cell death regulation (Yoshioka et al. 2011; Yu et al. 2012). In animal cells, nitric oxide synthase (NOS) was proposed to generate NO (Nathan and Xie 1994); however, neither homologs of animal NOS nor plant-specific NOS have been identified in plants (Guo et al. 2003; Crawford 2006). The distinct contribution of NO to cell death and resistance responses is not fully understood. However, it is suggested that ROS and NO are harmonized in the fine-tuned regulation of plant cell death and activation of innate immunity (Asai et al. 2008; Wang et al. 2013). Some pepper cell death-inducing genes, including *CaCaM1* (Choi et al. 2009), *CaPIK1* (Kim and Hwang 2011), *CaAS1* (Hwang et al. 2011), *CaADC1* (Kim et al. 2013a, 2013b) and *CaPLP1* (Kim et al. 2014a), induced ROS and an NO burst in plants. Notably, promoter analyses of *CaPIMP1* and *CaPR1* revealed their differential activation by NO in pepper leaves, suggesting the NO-

dependent transcriptional regulation of pepper HR cell death genes (Hong et al. 2005; Hong and Hwang 2009).

Reactive oxygen species (ROS) are responsible for mediating cellular cell death and defense responses in pepper plants. However, controversy has existed over the origin of ROS in plant defense (Choi et al. 2007). Redox-associated proteins such as peroxidase, ascorbate peroxidase, thioredoxin peroxidase and catalase have differentially been implicated in the generation of ROS during cell death and defense responses in *Xcv*–pepper interactions. Further studies are required to define the molecular mechanisms underlying the signaling pathways of ROS and NO bursts regulated by different redox-associated proteins in pepper plants.

Transcription factor (TF) proteins

Transcriptional regulation of defense gene expression is mediated by changes in the activity of DNA-binding transcription factors (TFs). Transcription factors contribute to the regulation of the plant defense response, including the up-regulation of the PR-genes, through recognition of specific DNA sequences in the promoter region (Rushton and Somssich 1998). HR cell death accompanies a genome-wide transcriptional response that is mediated by specific TFs. Some TF-encoding genes were isolated from pepper plants based on their pronounced expression pattern during HR cell death. The three *WRKY* TFs, *CaWRKY1*, *CaWRKY2* and *CaWRKY-a*, are strongly induced during AvrBs2-induced HR in peppers with the *Bs2* gene (Oh et al. 2006, 2008; Park et al. 2006). VIGS of *CaWRKY1* in pepper leaves resulted in enhanced resistance against *Xcv* infection, suggesting that CaWRKY1 negatively regulates basal resistance (Oh et al. 2008). Other TF genes that are activated during HR cell death in pepper are *AT-hook-Like 1* (*CaATL1*; Kim et al. 2007b), *ERF/AP2-type pathogen and freezing tolerance related protein 1* (*CaPF1*; Yi et al. 2004), *Related to ABI3/VP1* (*CaRAV1*; Sohn et al. 2006), *Cys2/His2-type Zinc-Finger Protein 1* (*CaZFP1*; Kim et al. 2004), *basic Leucine Zipper 1* (*CabZIP1*; Lee et al. 2006), *Ethylene-Responsive Factor Like Protein 1* (*CaERFLP1*; Lee et al. 2004), *NAC1* [*No Apical Meristem* (NAM), *ATAF1, 2* and *CUP-Shaped Cotyledon* (*CUC2*) *1*] (*CaNAC1*; Oh et al. 2005) and *CCR4-Associated Factor 1* (*CaCAF1*; Sarowar et al. 2007) (Table 1). Overexpression of *CaATL1* significantly enhanced the resistance of tomato plants against the bacterial pathogen *Pst* and the oomycete pathogen *P. capsici*. The ERF/AP2-type TF *CaPF1* shows distinct expression during HR and is implicated in tolerance to multiple biotic and abiotic stresses (Yi et al. 2004). Transgenic overexpression of *CaPF1* significantly enhanced the resistance of Arabidopsis plants against *Pst* infection, accompanied by constitutive up-regulation of *PR* and cold-regulated (*COR*) genes. Further studies using transgenic pine trees (*Pinus strobus* L.) overexpressing *CaPF1* revealed that *CaPF1*-induced multiple stress tolerance is associated with polyamine (PA) biosynthesis (Tang et al. 2007). Overexpression of *CaRAV1*, *CaZFP1* and *CabZIP1* enhanced basal resistance of Arabidopsis plants against *Pst* (Kim et al. 2004; Lee et al. 2006; Sohn et al. 2006). Notably, transgenic Arabidopsis plants overexpressing *CabZIP1* and *CaZFP1* showed a dramatic dwarf phenotype. *CaERFLP1* shows the highest homology with tomato *LeERF2* (73 %) and forms a specific complex with the GCC box and DRE/CRT motif (Lee et al. 2004). The sequences between −624 and −452 bp upstream of the *CARAV1* gene were shown to be essential for the *CARAV1* promoter activation by bacterial infection and SA treatment (Sohn et al. 2006). Two GCC box and 4 MYB binding sites within the *CARAV1* promoter region may be involved in the *P. syringae* pv. *tabaci* and SA-induced promoter activation. Transgenic tobacco plants overexpressing *CaERFLP1* exhibited significantly reduced disease lesions and leaf bacterial numbers after virulent *P. syringae* pv. *tabaci* infection. The function of *CaCAF1* was extensively studied using VIGS and transgenic approaches in pepper and tomato plants, respectively (Sarowar et al. 2007). Interestingly, VIGS of *CaCAF1* compromised avirulent *Xanthomonas*-induced HR cell death in pepper, suggesting that *CaCAF1* directly functions in cell death-mediated resistance. *CaNAC1* showed a specific expression pattern during pathogen-induced cell death; however, its role in cell death or disease resistance remains to be determined (Oh et al. 2005).

A variety of plant transcription factors play essential roles in the regulation of cell death and defense responses in pepper plants. In conclusion, the roles of CaZFP1 (Kim et al. 2004), CaRAV1 (Sohn et al. 2006) and CabZIP1 (Lee et al. 2006) in plant defense against pathogen infection, as a transcriptional activator, and its functional involvement in regulating expression of some PR genes have been demonstrated in 'gain-of-function' transgenic Arabidopsis plants. However, the target genes that bind to the TFs remain to be identified. Apparently, more detailed in vitro and in vivo analyses, such as loss-of-function, *trans* factor–*cis* element interaction and knockout mutation are also required to elucidate the roles of the TFs in the pepper defense cascades.

Secondary metabolite-associated proteins

Secondary metabolite biosynthesis plays an important role in the interactions of these plants with their environments (Pichersky and Gang 2000). For example, secondary metabolites aid plant fitness by preventing pathogen invasion and insect herbivory as well as by attracting

pollinators and natural enemies of herbivores (Wink 1998; Osbourn et al. 2003; Kliebenstein 2004). The plant kingdom produces approximately 50,000 secondary metabolites of known structures, including terpenoids, alkaloids, phenylpropanoids and other compounds (De Luca and St Pierre 2000).

The most well-known secondary metabolites of pepper plants include the alkaloid capsaicin and the terpenoid capsidiol. Biosynthesis of capsaicin is restricted to the genus *Capsicum* and is responsible for its characteristic pungency. The *Pun1* gene encodes a putative acyltransferase that is responsible for capsaicin biosynthesis and has a tissue-specific expression pattern (Stewart et al. 2005; Kim et al. 2009). Interestingly, capsaicin induces apoptosis in several animal cancer cells, such as pancreatic and stomach cancer cells (Sánchez et al. 2006; Wiwanitkit 2012). However, the role of capsaicin in cell death and disease resistance in pepper is not understood. Capsaicin is postulated to act as a deterrent to protect the pepper fruit and seeds from certain herbivores owing to its characteristic burning sensation (Tewksbury and Nabhan 2001). As a well-known pepper secondary metabolite, production of capsidiol, an antifungal sesquiterpenoid phytoalexin, is up-regulated in response to pathogenic and nonpathogenic fungi as well as to the systemic fungicide metalaxyl (Hwang and Sung 1989; Hwang et al. 1990). Importantly, metalaxyl-induced capsidiol production occurs in a dose-dependent manner and the levels are correlated with suppression of *Phytophthora* blight disease in pepper plants.

Recent reverse genetic studies in the pepper–*Xanthomonas* pathosystem revealed that some pepper genes such as menthone reductase (*CaMNR1*), arginine decarboxylase 1 (*CaADC1*) and phenylalanine ammonia-lyase (*CaPAL1*) are involved in secondary metabolite production (Table 1). The pepper menthone, (+)-(3*S*)-neomenthol reductase gene *CaMNR1*, was up-regulated during HR cell death of pepper leaves infected with avirulent *Xcv* carrying *avrBsT* (Choi et al. 2008). Transgenic expression of *CaMNR1* enhanced the broad-spectrum resistance of Arabidopsis against the hemibiotrophic pathogen *Pst* and the biotrophic pathogen *Hyaloperonospora parasitica*. By contrast, *CaMNR1*-silenced pepper plants exhibited enhanced susceptibility to the hemibiotrophic bacterial pathogen *Xcv* and the necrotrophic fungal pathogen *C. coccodes*. CaMNR1 exhibited an enzymatic activity for menthone reduction to produce neomenthol in vitro, as predicted by its significant sequence identity with peppermint MNR (Davis et al. 2005). Interestingly, the neomenthol generated by CaMNR1 strongly inhibited phytopathogenic bacterial and fungal growth compared with its precursor form, menthone.

To investigate the cell death signaling induced by the *Xcv* effector AvrBsT, arginine decarboxylase 1 (*CaADC1*) was identified as an AvrBsT-interacting protein by yeast two-hybrid screening (Kim et al. 2013a). *CaADC1* was strongly induced in pepper leaves during the development of HR cell death induced by avirulent *Xcv* carrying *avrBsT*. In plants, the ADC enzyme catalyzes arginine decarboxylation to produce agmatine, which is further metabolized to produce putrescine (Bagni and Tassoni 2001; Walters 2003). Polyamines (PAs), such as putrescine and spermidine, can be catabolized to pyrroline, which is further processed to form γ-aminobutyric acid (GABA) (Flores and Filner 1985). GABA is proposed to contribute to defense responses by affecting the ROS burst during pathogen infection (Bouché and Fromm 2004). *Agrobacterium*-mediated transient expression of *CaADC1* significantly enhanced the accumulation of PAs and the radical burst in *N. benthamiana* leaves, thereby enhancing AvrBsT-triggered HR cell death (Kim et al. 2013a). By contrast, VIGS of *CaADC1* not only compromised avirulent *Xcv* (*avrBsT*)-induced HR cell death, but also reduced PA, GABA and SA levels in pepper leaves. Exogenous spermidine treatment mimics CaADC1-induced cell death accompanied by a radical burst. Together, PA-induced ROS and NO bursts and CaADC1-induced GABA synthesis are proposed to activate downstream signaling pathways, which regulate cell death and defense responses in pepper plants.

Phenylalanine ammonia-lyase (PAL) has a crucial role in secondary phenylpropanoid metabolism and is one of the most extensively studied enzymes with respect to plant responses to biotic and abiotic stress. CaPAL1 acts as a positive regulator of SA-dependent defense signaling to combat microbial pathogens via its enzymatic activity in the phenylpropanoid pathway (Kim and Hwang 2014). Reactive oxygen species (ROS), hypersensitive cell death, expression of the salicylic acid (SA)-dependent marker gene *CaPR1*, SA accumulation and induction of PAL activity were significantly compromised in the *CaPAL1*-silenced pepper plants during *Xcv* infection.

In pepper plants, the significance of secondary metabolites, such as capsaicin, capsidiol and polyamines has been demonstrated in pepper defense responses to biotic and abiotic stresses. Pepper plants may elaborate a vast array of enzymes that synthesize such defensive secondary metabolites in response to pathogen attack. The pepper menthone, (+)-(3*S*)-neomenthol reductase CaMNR1 (Choi et al. 2008), and arginine decarboxylase 1 CaADC1 (Kim et al. 2013a, b) have recently been proposed to act as key regulators of cell death and defense signaling in pepper plants. However, further precise investigations are required to better understand the functions of the enzymes and genes that control the biosynthesis of secondary metabolites related to cell death defenses in pepper.

Lipid metabolism-associated proteins

Lipid metabolism has recently been shown to play critical roles in plant defense responses (Munnik 2001). Lipids and lipid metabolites affect pathogenesis and resistance mechanisms associated with plant–microbe interactions. Metabolite profiling in extracts of pepper leaves inoculated with avirulent *Xcv* revealed the accumulation of the lipid-derived volatiles 2-hexenal and *cis*-3-hexenol, which exhibit antifungal and antiprotozoal activities in vitro (Choi et al. 2008). In addition, *Xcv*-induced HR cell death in pepper triggered de novo synthesis of *trans*-2-*cis*-6-nonadienal in pepper plants, which is consistent with the HR-specific accumulation of this compound in *P. vulgaris* leaves (Croft et al. 1993). *Trans*-2-*cis*-6-nonadienal is synthesized from the 9-hydroperoxide of linolenic acid by hydroperoxide lyase activity (Matthew and Galliard 1978). The absence of *trans*-2-*cis*-6-nonadienal in uninfected healthy or virulent bacterial pathogen-infected *P. vulgaris* leaves (Croft et al. 1993) and pepper leaves (Choi et al. 2008) suggests that a highly specific and common mode of lipid peroxidation occurs during the HR in plants.

Lipid peroxidation is an important hallmark of plant HR cell death. Plant lipoxygenases (LOXs) are key enzymes involved in the generation of fatty acid derivatives in oxylipin metabolism. The active lipid peroxidation initiated by LOXs is sufficient to induce plant HR cell death (Montillet et al. 2005). In pepper, HR cell death-specific lipid peroxidation is proposed to be controlled by pathogen- and ethylene-inducible *9-lipoxygenase* (*CaLOX1*) (Hwang and Hwang 2010). Transient expression of *CaLOX1* in pepper leaves induced the pathogen-independent cell death phenotype, suggesting its important role in cell death regulation. *CaLOX1*-silenced pepper plants were more susceptible than wild-type plants to *Xcv* and *C. coccodes* infection, which was accompanied by reduced expression of defense-related genes, reduced lipid peroxidation as well as decreased ROS and SA accumulation. The other lipid metabolism-associated genes, *GDSL-motif Lipases* (*CaGL1* and *CaGLIP1*; Hong et al. 2008b; Kim et al. 2008), *Fatty Acid Desaturase* (*CaFAD1*; Kim et al. 2007a), *Lipid Transfer Proteins* (*CaLTP1*, *2*, and *3*; Jung et al. 2003) and patatin-like phospholipase (*CaPLP1,* Kim et al. 2014a), were isolated and functionally characterized based on their specific expression during pathogen-induced cell death (Table 1). CaGLIP1 modulates disease susceptibility and abiotic stress tolerance (Hong et al. 2008b). Differential responses of the *CaGLIP1* transgenic plants to disease, drought, ABA, glucose and oxidative stress resulted from enhanced lipase activity in transgenic *Arabidopsis* plants. The *CaLTPI* and *CaLTPIII* genes, although different in sequence structure, are transcriptionally activated in pepper tissues by pathogen infection as well as abiotic and environmental stresses (Jung et al. 2003). Phospholipases hydrolyze phospholipids into fatty acids and other lipophilic substances. Phospholipid signaling is crucial for diverse cellular processes in plants. Patatin-like phospholipases (PLPs), which are induced in plants by infectious microbial pathogens, mediate the biosynthesis of oxylipins in plant innate immunity and play a vital role for the resistance-associated cell death response to pathogens (La Camera et al. 2009). CaPLP1 functions as a positive regulator to enhance plant innate immunity and the cell death response (Kim et al. 2014a).

In conclusion, lipid metabolism-associated proteins, such as lipoxygenases, lipases and lipid transfer proteins are suggested to function as physiological and molecular regulators of diverse plant metabolic pathways in pepper. Further detailed studies will be done to clarify how the lipid metabolism-associated proteins affect pathogen- and abiotic elicitor-induced signal networking in pepper plants under adverse environmental conditions.

Cell wall proteins

Plant cell walls provide a physical barrier that separates challenging pathogens from the internal contents of plant cells. Additionally, cell walls regulate cell expansion and differentiation (Flors et al. 2007; Cantu et al. 2008). Polysaccharides, such as cellulose, hemicellulose and pectic polysaccharides, are the main components of primary cell walls. The cell wall strengthens during HR cell death. Histochemical analyses of cells involved in pepper–*P. capsici* interactions demonstrate that reinforcement of the cell wall compartment is part of HR cell death-mediated resistance (Hwang et al. 1989).

Recent reports on the pepper *xyloglucan-specific endo-β-1,4-glucanase inhibitor-protein1* gene (*CaXEGIP1*) provide experimental evidence of the molecular mechanism that underlies cell wall strengthening and signaling during HR cell death (Choi et al. 2013c) (Table 1). Purified CaXEGIP1 protein inhibited the hydrolytic activity of the GH74 family member XEG from the thermophilic bacterium *Clostridium thermocellum*. Notably, VIGS of *CaXEGIP1* significantly enhanced the growth of virulent and avirulent *Xcv* in pepper leaves, accompanied by compromised HR cell death. Conversely, overexpression of CaXEGIP1 induced spontaneous cell death, *PR* gene expression and thickening of the cell wall compartment. These findings support the notion that CaXEGIP1 targets plant xyloglucan-modifying enzymes to initiate HR cell death and defense signaling. Further studies will be required to understand how CaXEGIP1 and hemicellulose xyloglucan regulate pepper defense and/or cell death during the pathogen infection. Recently, Yeom et al. (2011) screened pepper *Secretome* (*CaS*) genes encoding defense-

related extracellular proteins using yeast-based signal-sequence trap screening. From this screening, 101 unique pepper genes were identified and VIGS of several CaS genes compromised non-host pathogen *Pst*-induced HR cell death in pepper. Further studies on the pepper cell wall *Proline-Rich Glycoprotein 1* gene (*CaPRP1*) revealed that expression of this gene accelerates cell death, but down-regulates ROS-scavenging and *PR* genes (Yeom et al. 2012).

Proteins embedded in the cell wall and plasma membrane are involved in a monitoring system that is required for the recognition and transduction of environmental, developmental and defense-associated signals (Garcia-Brugger et al. 2006). These proteins are released from plants or pathogens, and pathogens can trigger cell wall-mediated defense responses. Plant cell walls are also involved in generation of defense signaling molecules or damage-associated molecular patterns (DAMPs), such as oligogalacturonides (OGs) (Ferrari et al. 2013). Partial degradation of the cell wall component homogalacturonan releases OGs that can elicit defense responses, including ROS burst and *PR* gene expression, thereby protecting plants against pathogen infections. However, the molecular mechanisms underlying cell wall strengthening and/or cell wall-mediated defense signaling during HR cell death in pepper are poorly understood.

Antimicrobial proteins

Antimicrobial peptides appear to be involved in constitutive or induced resistance to various pathogens. Their modes of action are varied and include fungal cell wall degradation, membrane channel and pore formation, inhibition of DNA synthesis, damage to cellular ribosomes and cell cycle inhibition (DeGray et al. 2001; Selitrennikoff 2001; Shatters et al. 2006).

In pepper plants, the non-classical PR proteins CaAMP1, CaSAR82, CaCBP1 and CaPMEI1, which do not belong to the classical PR proteins (e.g., PR-1, PR-2 and PR-3) (van Loon et al. 2006), reportedly exhibit in vitro antimicrobial activities (Table 1). Three different cDNAs encoding the non-classical PR protein CaSAR8.2 were identified and expressed locally and systemically in pepper plants treated with biotic and abiotic elicitors (Lee and Hwang 2003). Further transgenic and biochemical studies of the CaSAR8.2 protein revealed its antifungal activities against the tested fungi, including *Alternaria*, *Botrytis*, *Colletotrichum*, *Fusarium* and *Phytophthora*, but not against bacterial pathogens (Lee and Hwang 2006). Interestingly, transgenic expression of *CaSAR8.2* in Arabidopsis not only enhanced antifungal defense, but also enhanced abiotic stress tolerance. The pepper *Pectin Methylesterase Inhibitor Protein* gene *CaPMEI1* was strongly induced in pepper leaves during HR development upon avirulent *Xcv* infection (An et al. 2008b). Notably, CaPMEI1 protein purified from *E. coli* exhibited antifungal activity against *Fusarium oxysporum*, *Alternaria brassicicola* and *Botrytis cinerea*. Further studies on the promoter region of this gene using the *GUS* reporter gene revealed that *CaPMEI1* is required for biotic and abiotic defense responses (An et al. 2009). The novel *Antimicrobial Protein* gene *CaAMP1* was isolated from pepper leaves infected with *Xcv* (Lee et al. 2008). Recombinant CaAMP1 purified from *E. coli* completely inhibited the growth of various microorganisms, including bacterial and fungal phytopathogens at concentrations of 5–100 μg mL^{-1}. *CaAMP1*-silenced pepper plants showed enhanced susceptibility to *Xcv* and *C. coccodes* infection. Overexpression of *CaAMP1* in Arabidopsis conferred broad-spectrum resistance to *Pst*, *H. arabidopsidis, F. oxysporum* and *A. brassicicola*. Proteomics analyses of transgenic Arabidopsis plants overexpressing *CaAMP1* revealed that CaAMP1 not only enhances antimicrobial defense, but also ROS-scavenging systems at the protein level (Lee et al. 2011b).

The past decade has seen advances in understanding the involvement of the pepper antimicrobial protein genes, especially CaSAR8.2, *CaPMEI1* and *CaAMP1*, in plant cell death and defense responses to microbial pathogens. The antimicrobial protein gene, encoding a protein with a high antimicrobial activity, may represent a new prospective transgene for engineering disease resistance in crop plants. However, identification of the target pathways for the antimicrobial proteins will be required to gain insights into the plant cell death and defense mechanisms that may be mediated by the antimicrobial protein gene expression.

Cell death-associated proteins

Pepper defense response genes, such as *Leucine-Rich Repeat 1* (*CaLRR1*) (Jung et al. 2004; Jung and Hwang 2007; Choi et al. 2012), *Hypersensitive Induced Reaction 1* (*CaHIR1*) (Jung and Hwang 2007; Jung et al. 2008), *Bax Inhibitor-1* (*CaBI-1*) (Ishikawa et al. 2011), *ABA-Responsive 1* (*CaABR1*) (Choi and Hwang 2011a), *Metacaspase 9* (*Camc9*) (Kim et al. 2013b), *E3 Ubiquitin Ligase 1* (*CaRING1*) (Lee et al. 2011a), *RNA-Binding Protein 1* (*CaRBP1*) (Lee et al. 2012) and cysteine/histidine-rich DC1 domain protein (*CaDC1*) (Hwang et al. 2014a), positively or negatively regulate the induction of HR cell death in plants (Table 1). Leucine-rich repeat proteins (LRRs) function in a number of signal transduction pathways via protein–protein interactions (Jung et al. 2004; Jung and Hwang 2007). Plasma membrane-resident CaHIR1 is proposed to be a key regulator of HR cell death. CaHIR1 positively regulates cell death induction in pepper plants; however, its interacting partner, CaLRR1, negatively

regulates CaHIR1-induced cell death (Jung and Hwang 2007). Microscopic and biochemical studies of pepper and Arabidopsis HIR proteins revealed that HIR proteins are enriched in the plasma membrane microdomain (Choi et al. 2011; Qi et al. 2011; Qi and Katagiri 2012). Interestingly, Arabidopsis HIR proteins are physically associated with two different immune receptors, namely, resistance to *Pseudomonas syringae* 2 (RPS2) and flagellin-sensitive 2 (FLS2) (Qi et al. 2011; Qi and Katagiri 2012). More recently, the *Xcv* filamentous hemagglutinin (FHA1) protein was found to interact with CaHIR1 to induce disease-associated cell death, thereby enhancing the virulence of the pathogen (Choi et al. 2013b). Taken together, the CaHIR1-associated membrane microdomain seems to play an important role in the very early stage of the plant–pathogen interaction to induce immunity or susceptibility-associated cell death.

Virus-induced gene silencing (VIGS) and *Agrobacterium*-mediated transient expression of *CaABR1, CaRING1, CaRBP1* and *CaDC1* were performed in pepper plants. Transgenic Arabidopsis plants overexpressing *CaABR1, CaRING1, CaRBP1* and *CaDC1* were also generated to determine whether the gain of function of these genes is required for basal resistance to plant pathogens. Expression of *CaABR1* confers enhanced resistance to pathogen infection in pepper and transgenic Arabidopsis plants, which is accompanied by cell death, callose deposition and ABA–SA antagonism (Choi and Hwang 2011a). Ubiquitination is essential for ubiquitin/proteasome-mediated protein degradation. In plants, ubiquitination regulates endogenous signals in response to pathogen attack. CaRING1 is involved in the induction of cell death and the regulation of ubiquitination during the defense response to microbial pathogens (Lee et al. 2011a). RNA-binding proteins that recognize pre-mRNAs or mature mRNAs function as regulators of gene expression (Maris et al. 2005). *CaRBP1* induction in the cytoplasm leads to activation of cell death and defense responses (Lee et al. 2012). CaDC1 binds both RNA and DNA and functions as a positive regulator of plant cell death and SA-dependent defense responses (Hwang et al. 2014a). Taken together, these pepper genes are involved in cell death and defense signaling in plants.

Pathogenesis-related (PR) proteins

A variety of *PR* genes are induced in plant tissues upon pathogen infections, specifically during the HR. PR proteins are classified into more than 13 groups based on their structural homologies (van Loon et al. 2006). However, the biological and biochemical functions of these PR proteins during defense reactions and developmental processes are not fully understood. Similar to previous findings in various plant species, a variety of *PR* genes, such as *CaPR1* (Kim and Hwang 2000), *CaPR4b* (Hwang et al. 2014b), *CaPR10* (Choi et al. 2012), *CaOSM1* (Hong et al. 2004), *CaChi2* (Hong et al. 2000), *CaThi1* (Lee et al. 2000), *CaBGLU1* (Jung and Hwang 2000b), *CaCBP1* (Lee et al. 2001) and *CaDEF1* (Do et al. 2004), are significantly induced in pepper plants during HR cell death (Table 1). The pepper *PR* genes *CaPR5*/*CaOSM1* and *CaPR10* were recently shown to positively regulate HR cell death. Transient expression of plasma membrane-localized CaOSM1 protein induces pathogen-independent cell death in pepper leaves, which is accompanied by strong H_2O_2 accumulation (Choi et al. 2013a). CaPR10 is an interacting partner of CaLRR1 (Choi et al. 2012). More importantly, transient expression of *CaPR10* triggers HR cell death in pepper and *N. benthamiana* leaves, which is amplified by co-expression of *CaLRR1* as a positive regulator. CaLRR1 promotes the ribonuclease activity and phosphorylation of CaPR10, leading to enhancement of its cell death-inducing activity. By contrast, CaPR4b interacts with CaLRR1 to suppress CaPR4b-triggered cell death and defense in pepper plants (Hwang et al. 2014b). CaPR4b is synthesized in the endoplasmic reticulum (ER), interacts with CaLRR1 in the plasma membrane and is secreted to the apoplast via the plasma membrane.

Although the pepper PR proteins that trigger defense responses in plants have been well documented, there is little information available to describe how pepper PR proteins function in HR cell death and defense signaling. More detailed studies of the molecular mechanisms underlying how pepper PR proteins target the downstream defense response proteins in plants are needed.

Subcellular localizations of cell death response proteins

The specific subcellular localizations and translocations of cell death and defense-related proteins are important to trigger downstream cell death-mediated defense signaling in plants. For example, upon their activation, some cytosolic NB-LRR-type immune receptors enter and function in the nucleus (Sheen and He 2007; Shen and Schulze-Lefert 2007). Nuclear translocation of cytosolic fractions of Arabidopsis RPS4 (Wirthmueller et al. 2007) and tobacco N protein (Burch-Smith et al. 2007) are critical for their activation, ultimately leading to HR cell death. By contrast, potato Rx1 activates an antiviral mechanism in the cytoplasm, but not in the nucleus (Slootweg et al. 2010).

Differential subcellular localizations of several pepper defense-related proteins, such as ABA-responsive 1 (CaABR1) (Choi and Hwang 2011a), E3 ubiquitin ligase RING1 (CaRING1) (Lee et al. 2011a), CaPR10 (Choi et al. 2012), RNA-binding protein (CaRBP1) (Lee et al. 2012) and cysteine/histidine-rich DC1 domain protein 1 (CaDC1)

(Hwang et al. 2014a), play crucial roles in gene expression to trigger and regulate pathogen-independent HR cell death in plants (Table 1). Topology studies of these cell death response genes in combination with fusion of different signal peptide sequences are used to analyze the subcellular localizations of these proteins in plant cells. Full-length CaABR1 protein, which triggers cell death, specifically localized to the nucleus in plants (Choi and Hwang 2011a). Perturbation of the nuclear localization of CaABR1 by adding a nuclear export signal (NES) peptide compromised CaABR1-induced HR cell death, suggesting that the nuclear localization of CaABR1 is essential for it to trigger cell death in plants. Following transient expression of *CaRING1* in onion epidermal cells, CaRING1 localizes to the plasma membrane and its TM domain is essential for this subcellular localization (Lee et al. 2011a). These data suggest that localization of CaRING1 to the plasma membrane may be important for signal transduction of HR cell death during infection. The CaLRR1–CaPR10 complex forms in the cytoplasm and is secreted into the apoplastic space (Choi et al. 2012). The cytoplasmic CaLRR1–CaPR10 complex enhances CaPR10-triggered cell death in *N. benthamiana* leaves. The engineered nuclear confinement of both proteins revealed that the cytoplasmic localization of the CaPR10–CaLRR1 complex is essential for cell death-mediated defense signaling. However, translocation of CaPR4b from the endoplasmic reticulum (ER) to the apoplastic region is required for cell death induction (Hwang et al. 2014b). CaPR4b has a signal peptide required for ER localization and secretion. CaPR4b–GFP is visualized in the ER, plasma membrane and apoplastic region using a confocal microscope. The pepper cysteine/histidine-rich divergent C1 (DC1) domain protein CaDC1 can bind both DNA and RNA in vitro, specifically in the presence of Zn^{2+} (Hwang et al. 2014a). CaDC1 localizes to the nucleus and cytoplasm, which is required for plant cell death signaling. The nuclear localization of CaDC1 is dependent on its DC1 domain. However, the cytoplasmic localization of 35S:*CaDC1*^{c}, as well as the nuclear localization of 35S:*CaDC1-NLS*, was not sufficient to induce cell death in pepper leaves. Another pepper RNA-binding protein, CaRBP1, functions in HR cell death and defense signaling in the cytoplasm (Lee et al. 2012). Notably, cytoplasmic localization of CaRBP1, mediated by the N-terminal region of CaRBP1, is essential for the hypersensitive cell death response.

Certain plant cell death response proteins may act in specific subcellular locations to trigger downstream signaling and defense pathways. The pepper cell death response proteins investigated so far are localized to the specific cellular compartments, such as nucleus, ER, plasma membrane and cytoplasm, during the cell death and defense responses in plants. The significance of distinct subcellular localizations of these pepper cell death response proteins will be elucidated to better understand the molecular mechanisms underlying the downstream cell death signaling pathways in plant cells.

Hormonal control of cell death-mediated defense signaling

To cope with destructive diseases, plants have evolved molecular mechanisms mediated by multiple, highly regulated and coordinated signaling networks that rely on plant hormones, including salicylic acid (SA), jasmonic acid (JA), ethylene and abscisic acid (ABA), as secondary messengers (Kunkel and Brooks 2002; Grant and Jones 2009). Plant cell death and defense responses are controlled by the complex cross talk of these stress specific hormones. For example, JA is involved mainly in resistance to necrotrophic pathogens, which prefer to feed on dying tissues. On the other hand, SA is known to act as signaling molecules in plant defense against biotrophic pathogens, which feed mainly on living plant tissues. Comprehensively, experimental evidence supports the plant hormonal signaling pathways of cell death and defense responses in pepper.

Salicylic acid (SA) and jasmonic acid (JA)

Salicylic acid is a key regulator of plant defense responses (Vlot et al. 2009; Dempsey et al. 2011). In plants, direct application of SA or SA analogs induces *PR* gene expression and enhances disease resistance (Reignault and Walters 2007; Lee et al. 2009). SA-induced *PR* gene expression and disease resistance have been extensively investigated in pepper (Lee and Hwang 2005; Lee et al. 2009). Various cell death and defense response genes, such as *CaPR1*, *CaPR10*, *CaDEF1*, *CaSAR8.2*, *CaAMP1*, *CaPMEI1* and *CaLOX1* (Table 1), were strongly induced in pepper plants by exogenous SA treatment, and the gene expressions positively correlated with disease resistance. Notably, application of the functional SA analogs 2,6-dichloro isonicotinic acid and benzothiadiazole confers broad-spectrum resistance of pepper plants against various microbial pathogens (Choi and Hwang 2011b).

Despite the positive effect of SA on broad-spectrum resistance in pepper plants, the role of SA in the regulation of the cell death response seems to vary among the defense response genes. In many cases, endogenous SA accumulation positively correlates with HR cell death in pepper plants. For example, silencing of *CaRING1, CaRBP1* and *CaADC1* significantly compromised both SA accumulation and HR cell death induction in pepper plants inoculated with avirulent *Xcv* (Lee et al. 2011a, 2012; Kim et al. 2013a). By contrast, silencing of *CaHIR1* compromised

avirulent *Xcv*-induced HR cell death, but significantly enhanced SA accumulation (Choi et al. 2011). Notably, *CaHIR1*-silenced plants exhibited significantly enhanced expression of *PR* genes, including *CaPOA1*, which encodes an ROS-scavenging ascorbate peroxidase enzyme. This suggests that pre-existing SA in *CaHIR1*-silenced plants potentiates ROS-scavenging activity, thereby blocking avirulent *Xcv*-induced HR cell death. Exogenous treatment of pepper plants with SA induced the expression of different peroxidase genes, namely, *CaPOA1* and *CaPO1* (Do et al. 2003). Transcription of *CaPOA1* was strongly activated in pepper leaves by SA treatment at 18–24 h when the HR occurred in the incompatible interaction with *Xcv,* suggesting the involvement of *CaPOA1* in the triggering of HR cell death. CaRING1 is involved in cell death and the SA-dependent defense response in pepper (Lee et al. 2011a). The decrease in free SA in *CaRING1*-silenced leaves enhances plant susceptibility to avirulent Xcv infection in pepper. Thus, CaRING1 may not modulate the production of SA synthesis, but rather the conversion of SA to glucoside-conjugated SA (SAG), since the accumulation of total SA was similar in CaRING1-silenced and empty vector control plants. More recently, it has been demonstrated that *CaDC1* (Hwang et al. 2014a), *CaMBL1* (Hwang and Hwang 2011), *CaPO2* (Choi et al. 2007), *CaPIK1* (Kim and Hwang 2011) and *CaAS1* (Hwang et al. 2011) contribute positively to the regulation of SA- and ROS-mediated defense signaling, thereby activating HR cell death and disease resistance. The onset of systemic acquired resistance (SAR) is closely associated with a local and systemic increase in endogenous SA levels (Métraux et al. 1990; Vlot et al. 2009). The potential of SA produced during the HR contributes positively to the enhancement of HR cell death, further leading to the establishment of SAR.

The pepper RNA-binding protein *CaRBP1* expression is required for accumulation of SA and JA in *Xcv*-infected pepper plants (Lee et al. 2012). The enhanced disease susceptibility of the *CaRBP1*-silenced plants results in lower SA and JA synthesis. Plant inducible immunity employs the SA and JA pathways to defend against biotrophic and necrotophic pathogens, respectively (Tsuda et al. 2009). Expression of *CaPR1,* a SA-responsive marker gene, was greatly compromised in *CaRBP1*-silenced pepper plants following avirulent *Xcv* infection (Lee et al. 2012). Furthermore, the induction of *CaDEF1*, a JA-responsive marker gene, was also strongly suppressed in *CaRBP1* silenced pepper plants after avirulent *Xcv* infection. Collectively, these results suggest that *CaRBP1* may positively regulate the R gene-mediated resistance response associated with the SA- and JA-dependent signaling pathways. These hormonal signals are not linear, but establish complicated cross-talking networks. The plant hormones SA and JA may act independently or cooperatively for defense gene expression in pepper.

Ethylene (ET) and jasmonic acid (JA)

The plant hormones ET and JA are important defense signal compounds, which mediate defense signaling against infection by microbial pathogens in host plants. JA is involved mainly in resistance to necrotrophic pathogens, which prefer to feed on dying tissues. In *Arabidopsis*, JA- and ethylene-dependent defense signaling networks were demonstrated to modulate resistance to the necrotroph *B. cinerea* (Penninckx et al. 1998; Thomma et al. 1999). ET and JA act either independently or cooperatively to induce resistance and defense-related gene expression. In addition to these synergistic interactions, JA and/or ET can interact antagonistically with SA (Grant and Jones 2009). In pepper plants, exogenous application of ET or JA induces the expression of various defense-related genes encoding PR1 (CaPR1) (Kim and Hwang 2000), β-1,3-glucanase (CaBGLU1) (Jung and Hwang 2000b), chitinase (CaChi2) (Hong et al. 2000), thionin (CaThi1)(Lee et al. 2000), lipid transfer proteins (CaLTPs: CaLTPI, II, and III) (Jung et al. 2003, 2005), chitin-binding protein (CaCBP1) (Lee et al. 2001) and 9-lipoxygenase (CaLOX1) (Hwang and Hwang 2010). The pepper pectin methylesterase inhibitor (*CaPMEI1*) promoter regions contain positive ethylene- and MeJA-responsive *cis*-acting elements (An et al. 2009). *CaPMEI1* expression is induced in pepper leaves by infection with bacterial pathogens and treatment with plant hormones such as SA, ethylene, metyl jasmonic acid (MeJA) and ABA (An et al. 2008b). *CaPMEI1* promoter activation is suggested to be a critical molecular event for host defense response and ethylene- and MeJA-mediated CaPMEI1 gene expression (An et al. 2009). However, the role of ET and JA in the regulation of HR cell death in pepper plants is not fully understood.

Abscisic acid (ABA) and salicylic acid (SA)

Abscisic acid regulates various aspects of plant growth and development and is a critical signaling molecule to activate disease resistance in different plant species (Fan et al. 2009). ABA signaling plays an important role in seed development and tolerance to abiotic stresses, such as drought and high salinity. For example, bZIP-type AREB/ABF TFs control the promoter regions, designated ABA-responsive elements (ABREs), of stress-responsive genes to induce their expression (Yoshida et al. 2010). Compared to the role of ABA in plant abiotic stress tolerance, the role of ABA in disease resistance is largely unknown. ABA reportedly has an antagonistic effect on SA- and JA/ET-mediated defense signaling in Arabidopsis plants

(Anderson et al. 2004; Mauch-Mani and Mauch 2005). Exogenous ABA treatment enhances the susceptibility of rice to *Magnaporthe oryzae* (Koga et al. 2004). Necrotrophic fungal pathogens, such as *B. cinerea* and *F. oxysporum*, are reported to directly produce ABA (Marumo et al. 1982; Mauch-Mani and Mauch 2005). The *P. syringae* effector protein AvrPtoB stimulates ABA production, thereby hijacking the host ABA signaling network to modify the hormone balance in favor of the pathogen (de Torres-Zabala et al. 2007).

Several pepper HR cell death genes, such as *CaZFP1*, *CabZIP1*, *CaRAV1*, *CaCaM1*, *CaPO2*, *CaHIR1* and *CaABR1*, are strongly induced by exogenous ABA treatment (Table 1). Our data revealed that ABA and SA have antagonistic functions in *Xcv*–pepper interactions (Choi and Hwang 2011a, b). Increased ABA levels, together with SA reduction, were apparently observed in *CaABR1*-silenced pepper leaves. To resist bacterial pathogen attack, *CaABR1* expression may regulate and fine-tune endogenous ABA levels. *CaABR1* is rapidly and strongly induced by ABA treatment, but not SA treatment. *CaABR1* expression may enhance SA biosynthesis to trigger defense responses in pepper. Importantly, endogenous ABA levels enhanced by *CaABR1* silencing suppressed the SA-dependent marker gene *CaPR1* in pepper leaves infected with an avirulent strain of *Xcv*. Ectopic expression of *CaABR1* in *Arabidopsis* strongly supported the role of CaABR1 for basal and HR-like resistant responses to bacterial challenge. In other studies, the extracellular peroxidase gene *CaPO2* is strongly induced by ABA, but not by the defense-related plant hormone SA (Choi et al. 2007). Transgenic overexpression of *CaPO2* in *Arabidopsis* enhances not only broad-spectrum resistance, but also abiotic stress tolerance (Choi and Hwang 2012). Taken together, these studies suggest that interplay and antagonism between ABA and SA are crucial for basal and induced resistance in pepper plants. ABA accumulation is proposed to suppress SA-dependent defense signaling mechanisms, ultimately leading to basal susceptibility to bacterial and fungal pathogens in plants (Audenaert et al. 2002; Mohr and Cahill 2007). The relationship between SA and ABA reflects early host–pathogen conflict and modulates plant defense responses (de Torres-Zabala et al. 2007).

Disease-associated cell death in pepper

Disease-associated cell death is involved in host–pathogen compatible interactions that do not induce a resistance response. Cell death occurs in susceptible plants late during infection, which is usually caused by pathogen proliferation or by various toxins secreted by the pathogen. An increasing body of evidence suggests that host-controlled cell death is intimately linked with the onset of disease-associated cell death and symptom development in plants (Navarre and Wolpert 1999; Yao et al. 2002; Greenberg and Yao 2004; Choi et al. 2011). Although the timing and number of dying cells differ between resistant and susceptible responses, features of apoptotic cell death are observed in both. This strongly supports the notion that a common pathway underlying host-controlled cell death is activated in both compatible and incompatible plant-pathogen interactions. However, the molecular components regulating disease-associated cell death are poorly understood in plants.

Disease-associated cell death genes

In compatible plant–microbe interactions, disease-associated cell death occurs relatively late during the course of infection. Some pepper cell death response genes, such as *CaMLO1*, *CaMLO2*, *CaMRP1*, *CaGLIP1* and *CaHIR1*, have been identified as disease-associated cell death and susceptibility genes (Table 2). Silencing of *CaMLO1*, *CaMLO2* and *CaHIR1* compromised pathogen-induced cell death, but enhanced disease resistance in pepper plants. Expression of *CaMLO2* was up-regulated by *Xcv* infection and SA treatment (Kim and Hwang 2012). Silencing of *CaMLO2* in pepper plants confers enhanced resistance against virulent *Xcv*, accompanied by a compromised susceptibility cell death response, which suggests that *CaMLO2* is involved in disease-associated cell death. Zheng et al. (2013) reasoned that the expression of different MLO orthologs including *CaMLO1* and *CaMLO2* enhances susceptibility to the powdery mildew disease caused by *Leveillula taurica*. *Capsicum annuum metacaspase 9* (*Camc9*)-silenced pepper showed enhanced disease resistance, which was accompanied by suppressed disease-associated cell death (Kim et al. 2013b). By contrast, transgenic expression of *Camc9* in *N. benthamiana* enhanced disease-associated cell death upon virulent *P. syringae* pv. *tabaci* infection. These results suggest that *Camc9* is a positive regulator of disease-associated cell death in plants. Notably, *CaHIR1* positively regulates disease-associated cell death in pepper plants (Choi et al. 2011). During the compatible interaction of pepper plants with virulent *Xcv*. CaLRR1 and CaHIR1 proteins may be induced by PTI (Choi et al. 2011). Expression of *CaLRR1* contributes to the establishment of basal defense through SA-dependent signaling pathways. Induction of *CaHIR1* triggers cell death signals, in which signal intensities are not strong enough to exceed the threshold of the HR in the compatible interaction. Consequently, the signals triggered by CaHIR1 may lead to disease-associated cell death during compatible interactions. Silencing of *CaHIR1* in pepper enhanced resistance against virulent and avirulent *Xcv*

Table 2 Roles of pepper cell death and defense response genes in cell death and disease resistance, as evaluated by *Agrobacterium*-mediated transient expression, VIGS or transgenic overexpression

Gene name	Cell death[a]	VIGS plant[b]	Challenging pathogen[c]	Disease resistance[d]
CaRLK1	ND	ND	*P.syringae* pv. *tabaci*	+
			P. syringae pv. *tomato*	ND
CaPIMP1	ND	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	–
CaMRP1	ND	*Xcv* (+)	*H. arabidopsidis*	–
CaMLO1	+	*Xcv* (+)	*P. syringae* pv. *tomato*	–
and *CaMLO2*		*L. taurica* (+)	*H. arabidopsidis*	–
CaMBL1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			A. brassicicola	NC
CaPIK1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
CaCaM1	+	ND	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
			A. brassicicola	–
CaPO2	ND	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
			A. brassicicola	NC
CaMsRB2	ND	*Xav* (−)	*P. capsici*	+
			P. infestans	+
CaATL1	ND	ND	*P. capsici*	+
CaCAF1	ND	*Xav* (−)	*P. infestans*	+
		HR (−)		
CaMNR1	ND	*Xcv* (−)	*P. syringae* pv. *tomato*	+
		C. coccodes (−)	*H. arabidopsidis*	+
CaADC1	AvrBsT*	*Xcv* (−)	ND	ND
CaGLIP1	ND	Xcv (+)	*H. arabidopsidis*	–
CaLOX1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
		C. coccodes (−)	*H. arabidopsidis*	+
CaXEGIP1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	NC
			H. arabidopsidis	+
CaAMP1	ND	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
		C. coccodes (−)	*A. brassicicola*	+
			F. oxysporum	+
CaPMEI1	ND	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	NC
CaLRR1	±	*Xcv* (NC)	ND	ND
CaHIR1	+	*Xcv* (+)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
CaABR1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
Camc9	ND	*Xcv* (+)	*P. syringae* pv. *tomato*	–
CaRBP1	+	*Xcv* (−)	*H. arabidopsidis*	+
CaRING1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
CaPR10	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+
CaOSM1	+	*Xcv* (−)	*P. syringae* pv. *tomato*	+
			H. arabidopsidis	+

ND not determined, *NC* not changed, (+) positive cell death, (−) negative cell death, *CaADC1 AvR** positively regulated *Xcv* effector AvrBsT-triggered HR cell death, *CaLRR1* (±) positively regulated cell death induced by *CaPR10*, but negatively regulated cell death induced by *CaHIR1*, *Bax* and *avrPto/Pto*

[a] Induction of cell death in pepper or *Nicotiana benthamiana* by *Agrobacterium*-mediated transient expression

[b] Disease resistance of gene-silenced plants against the indicated pathogens

[c] Transgenic overexpression studies performed in *Arabidopsis thaliana*, except *CaRLK1* (in *N. benthamiana*), *CaMsRB2* (in *S. lycopersicum*), *CaATL1*(in *S. lycopersicum*), *CaCAF1* (in *S. esculentum*) and *Camc9* (in *N. benthamiana*)

[d] Disease resistance of transgenic plants. Enhanced resistance and susceptibility against pathogens are shown by (+) and (−), respectively

infection, which was accompanied by increased SA accumulation. A recent yeast two-hybrid screening using CaHIR1 as the bait identified the *Xanthomonas* filamentous hemagglutinin-like protein (*Xcv* Fha1) as a CaHIR1-interacting partner (Choi et al. 2013b). *Xcv* Fha1 is a virulence factor in host plants and is required to enhance the induction of disease-associated cell death by CaHIR1.

Engineering disease resistance using pepper cell death and defense response genes in plants

The conventional breeding program to generate disease-resistant plants mostly depends on the introgression of a dominant *R*-gene locus from wild-type species into the cultivated crop plants. This is because *R*-gene-mediated resistance (or ETI) provides the highest level of protection against plant diseases (Stuiver and Custers 2001; Wally and Punja 2010). However, *R*-gene-mediated resistance cannot be solely used for the breeding program owing to its breakdown by new virulent mutant races of the pathogen. Pathogens often overcome plant *R*-gene-mediated resistance by activating the corresponding effector gene (Stall et al. 2009). To resolve this resistance breakdown problem, some pepper HR cell death-associated genes are available as an alternative strategy to breed disease-resistant plants. Importantly, the expression of cell death genes mimics HR cell death in plants, even in the absence of the recognition of Avr proteins by the corresponding R proteins (without ETI activation). Conditional overexpression of cell death genes in pepper plants using specific promoters triggers HR cell death, thereby increasing disease resistance. However, there are some points to be addressed before this strategy is used. First, cell death gene-mediated downstream signaling should be more comprehensively understood. Increasing evidence suggests that pathogen effectors actively divert cellular signaling pathways of the host to the benefit of the pathogen (Kim et al. 2010). The cell death gene-mediated defense signaling pathway could not be readily suppressed by specific pathogen effectors, compared to the *R*-gene-mediated resistance signaling pathway. Overexpression of pepper cell death response genes in Arabidopsis often induces downstream defense signaling genes (Choi et al. 2008, 2009; Kim et al. 2013a). These downstream defense signaling genes may contribute positively to the HR cell death and defense responses associated with the cross talk between plant hormones, such as salicylic acid, jasmonic acid and abscisic acid (Choi and Hwang 2011a; Lee et al. 2011a, b). Transgenic Arabidopsis plants overexpressing *CaRING1* exhibit enhanced bacterial and fungal disease resistance, which as accompanied by the induction of SA-responsive *AtPR1* and ethylene/jasmonic acid (JA)-responsive *AtPDF1.2* (for Plant Defensin1.2) during *P. syringae* pv. *tomato* infection (Lee et al. 2011a). Second, overexpression of cell death genes in transgenic plants may be under the tight control of pathogen-inducible promoters. Constitutive expression of cell death genes may cause a constitutive HR cell death response in plants, thereby interfering with normal plant development and growth (Mittler and Rizhsky 2000). For example, constitutive overexpression of some pepper cell death genes under the CaMV 35S promoter in *Arabidopsis* often induces spontaneous cell death and reduces plant normal growth (Choi et al. 2009, 2013a). The combinational use of the promoters fitted for cell death genes that are specifically activated during the pathogen infection will be required to avoid the adverse effects of cell death genes on plant development. Third, the effects of cell death genes may vary among different plant pathogens. In fields, crop plants are challenged by a wide range of pathogens that have diverse lifestyles. For example, overexpression of the pepper cell death gene *CaCaM1* strongly enhanced resistance of Arabidopsis plants against the biotrophic pathogen *H. arabidopsidis* and the hemibiotrophic pathogen *Pst*, but reduced resistance against the necrotrophic pathogen *Alternaria brassicicola* (Choi et al. 2009). Thus, whether the introduction of the cell death gene into a certain plant affects pathogens of different lifestyles in different ways should be fully investigated. Finally, pepper cell death genes can be used as a genetic source for molecular breeding of disease-resistant strains of other plant species. Notably, transgenic overexpression of *CaPR1* and *CaLTP1* in tobacco plants led to enhanced resistance to some microbial pathogens, including *Phytophthora nicotianae*, *P. syringae* pv. *tabaci* and *Ralstonia solanacearum* (Sarowar et al. 2005, 2009). However, these transformed crops resistant to plant diseases should be more precisely investigated to evaluate if they are durably resistant to diverse plant diseases in the field.

Disease-associated cell death genes, such as barley *mlo*, Arabidopsis *powdery mildew-resistant6* (*pmr6*) and pepper *eukaryotic translation initiation factor 4E* (*eIF4E*) (Eckardt 2002; Kang et al. 2007; Pavan et al. 2010), are available for use in alternative strategies to breed disease-resistant plants. Loss-of-function mutants of these genes conferred durable and broad-spectrum disease resistance in different plant species. Notably, a recessive *mlo* mutation confers durable resistance of barley to powdery mildew (*Blumeria graminis* f.sp. *hordei*) for more than 30 years (Humphry et al. 2006). Transgenic tomato plants overexpressing the pepper mutant *eIF4E* showed dominant resistance to several tobacco etch virus strains and other potyviruses, including pepper mottle virus. Interestingly, some disease-associated cell death pepper genes, including *CaMLO1*, *CaMLO2*, *CaHIR1* and *CaRLK1*, strongly trigger cell death, but negatively regulate disease resistance

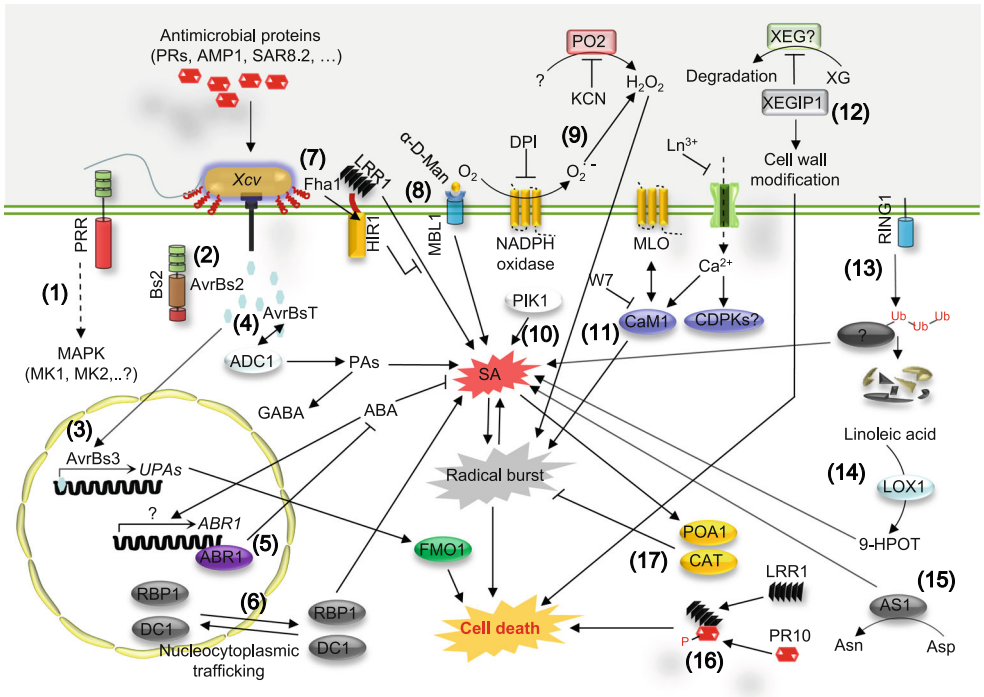

Fig. 2 Molecular and biochemical basis of signaling pathways underlying cell death and defense responses in pepper–*X. campestris* pv. *vesicatoria* (*Xcv*) interactions. *1* Pattern recognition receptor (*PRR*) signaling is poorly understood in pepper plants. *2* The *Xcv* type III secretion effector AvrBs2 recognizes pepper Bs2, leading to hypersensitive response (*HR*). *3* AvrBs3 translocates into the nucleus and induces the expression of *up-regulated by AvrBs3* gene (*UPAs*), including *FMO1*, which induces HR cell death in pepper. *4* AvrBsT interacts with arginine decarboxylase (*ADC1*), which regulates polyamine (*PA*)-mediated cell death and defense response. *5* Accumulation of the abscisic acid (*ABA*)-responsive protein ABR1 reduces the endogenous level of ABA. *6* Cytoplasmic RNA-binding protein (*RBP1*) or DC1 domain protein (*DC1*) localized in the nucleus and cytoplasm is required for cell death signaling. *7* *Xcv* filamentous hemagglutinin (*Fha1*) interacts with hypersensitive induced reaction protein (*HIR1*) and activates disease-associated cell death. HIR1 suppresses LRR1-mediated defense signaling. *8* Mannose (*Man*)-binding lectin (*MBL1*) is implicated in the recognition of pathogen-derived glycoproteins with Man residues. *9* Extracellular peroxidase (*PO2*) and NADPH oxidase are required for pathogen-triggered reactive oxygen species generation and HR cell death. *10* Receptor-like cytoplasmic protein kinase (*PIK1*) positively regulates salicylic acid (*SA*) accumulation and radical burst, thereby enhancing cell death and disease resistance. *11* Pathogen-triggered cytosolic calcium influx activates a calmodulin (*CaM1*)-dependent radical burst. The function of calcium-dependent protein kinases (*CDPKs*) in the HR is unknown in pepper. *12* Xyloglucanase inhibitor (*XEGIP1*) protein specifically accumulates and modifies plant cell wall structures to induce cell death. *13* Plasma membrane-localized RING1 domain-containing E3 ubiquitin ligase (*RING1*) is proposed to ubiquitinate unknown target proteins and thereby trigger their degradation, which triggers SA-dependent signaling during HR cell death. *14* 9-Lipoxygenase (*LOX1*) catalyzes hydroperoxidation of linoleic acid to produce 9-HPOT. *15* Asparagine synthetase (*AS1*) positively regulates nitrogen assimilation and SA-dependent defense signaling. *16* The cytosolic LRR1–PR10 complex induces cell death. *17* SA-inducible ascorbate peroxidase (*POA1*) and catalase (*CAT*) negatively regulate cell death by detoxifying ROS. Expression of (4) *ADC1*, (5) *ABR1*, (6) *RBP1*, (6) *DC1*, (7) *LRR1*, (8) *MBL1*, (10) *PIK1*, (13) *RING1*, (14) *LOX1*, (15) *AS1* and (16) *PR10* induce SA accumulation and cell death in pepper plants. *Asp* aspartate, *Asn* asparagine, *DPI* diphenylene iodonium as an NADPH oxidase inhibitor, *GABA* gamma-aminobutyric acid, *9-HPOT* 9-hydroperoxylinolenic acid, *KCN* potassium cyanide as a peroxidase inhibitor, La^{3+} lanthanum ion as a calcium channel blocker, *PAs* polyamines, *W7* *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide as a calmodulin agonist

(Table 2). Silencing of these susceptibility cell death genes significantly reduced the development of disease symptoms, as well as pathogen growth. Therefore, silencing of disease-associated cell death genes can be used as an alternative strategy to develop disease-resistant plants.

Conclusions and future prospects

Plants have evolved sophisticated molecular and cellular mechanisms to protect themselves against various pathogen attacks. Much has been learned during the last decades regarding the molecular and cellular basis of pepper cell death-mediated defense responses. Pepper cell death and defense response genes are functionally characterized in plants using *Agrobacterium*-mediated transient expression, VIGS and transgenic overexpression techniques. Notably, some defense response genes induce pathogen-dependent or -independent cell death in plants. This information can be useful to develop transgenic disease-resistant plants. Given that a small number of pathogens cause severe damage to a certain crop, positive or

negative regulation of the cell death pathway in certain plant species may have a huge impact on the control of major plant diseases.

Virus-induced gene silencing (VIGS) has recently been used to define the loss of function of pepper cell death- and defense-related genes. However, the lack of information on the pepper genome sequence makes it difficult to elucidate the exact functions of each gene family. Comprehensive functional studies of pepper cell death genes based on the full genome sequence information are required to understand how pepper plants respond to different types of pathogens by regulating cell death responses (Kim et al. 2014d). Based on the cell death research data of pepper, a network of defense signaling events from recognition of *X. campestris* pv. *vesicatoria* (*Xcv*) to cell death-mediated resistance in pepper plants is depicted in Fig. 2. None of the pattern recognition receptors (PRRs) or their cognate pathogen-associated molecular patterns (PAMPs) is yet identified from pepper plants. However, *Xanthomonas* type III effector proteins AvrBs2, AvrBs3 and AvrBsT are secreted into the host cell to mediate the effector-triggered immunity (ETI) signaling of pepper. AvrBs2 recognizes cytosolic nucleotide-binding site leucine-rich repeat (NBS-LRR) class R protein Bs2 to trigger the hypersensitive response (HR) in pepper leaves. AvrBs3 translocates into the nucleus and transcriptionally activates the set of host genes named *up-regulated by AvrBs3* (*UPAs*), including *FMO1,* which leads to the HR cell death in pepper. The cognate R protein of AvrBsT is not reported in pepper; however, the pepper arginine decarboxylase (CaADC1) is identified as an interacting partner of AvrBsT (Kim et al. 2013a). CaADC1 positively regulates polyamine (PA)-mediated cell death and defense response against *Xanthomonas* strain carrying *avrBsT*. A number of cell death-associated genes were identified and functionally characterized from pepper leaves infected with avirulent *Xcv* Bv5-4a carrying *avrBsT*. Notably, most of the cell death and defense response genes, such as *CaRBP1* (Lee et al. 2012), *CaDC1* (Hwang et al. 2014a), *CaMBL1* (Hwang and Hwang 2011), *CaPO2* (Choi et al. 2007), *CaPIK1* (Kim and Hwang 2011), *CaRING1* (Lee et al. 2011a), *CaLOX1* (Hwang and Hwang 2010) and *CaAS1* (Hwang et al. 2011), contribute positively to the regulation of SA- and ROS-mediated defense signaling, thereby activating HR cell death and disease resistance. High levels of abscisic acid (ABA) in *CaABR1*-silenced pepper plants antagonize the SA levels induced by *Xcv* infection (Choi and Hwang 2011a). The nuclear ABR1 pool is essential for cell death induction associated with ABA–SA antagonism. *Xcv* filamentous hemagglutinin (Fha1) interacts with hypersensitive induced reaction protein (CaHIR1) and activates disease-associated cell death (Choi et al. 2013b). CaHIR1 suppresses CaLRR1-mediated defense signaling (Jung and Hwang 2007). Pathogen-triggered cytosolic calcium influx activates a calmodulin (CaM1)-dependent radical burst (Choi et al. 2009). The function of calcium-dependent protein kinases (CDPKs) in the HR is unknown in pepper. Xyloglucanase inhibitor (CaXEGIP1) protein specifically accumulates and modifies plant cell wall structures to induce cell death (Choi et al. 2013c). Plasma membrane-localized RING1 domain-containing E3 ubiquitin ligase (CaRING1) is proposed to ubiquitinate unknown target proteins and thereby trigger their degradation, which triggers SA-dependent signaling during the HR cell death (Lee et al. 2011a). The cytosolic CaLRR1–CaPR10 complex is responsible for cell death-mediated defense signaling (Choi et al. 2012). By contrast, CaLRR1 interacts with PR4b to suppress PR4b-triggered cell death and defense response in pepper (Hwang et al. 2014b). SA-inducible ascorbate peroxidase (POA1) and catalase (CAT) negatively regulate cell death by detoxifying ROS. Further comprehensive understanding of the molecular and cellular basis of HR cell death in pepper will provide new insights into how pepper plants appropriately respond to a wide array of pathogens, which will ultimately lead to the establishment of a sustainable disease management strategy.

In addition, the expression of some cell death genes in pepper plants is closely related to various abiotic stress tolerances (Hong et al. 2008b; An et al. 2008b; Lee et al. 2011b; Choi and Hwang 2012). Accordingly, the pepper cell death response may positively regulate environmental stress in nature during plant growth. Therefore, it would be interesting to investigate whether the overexpression of pepper cell death genes in various plants can trigger abiotic stress tolerance.

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