Mini-Review

Roles of Plant Proteases in Pathogen Defense

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The genomes of plants contain more than 600 genes encoding a diverse set of proteases and the subunits of proteasomes. These proteases and proteasomes consist of plant proteolytic systems, which are involved in various cellular metabolic processes. Plant proteolytic systems have been shown to have diverse roles in defense responses, such as execution of the attack on the invading organisms, participation in signaling cascades, and perception of the invaders. In order to provide a framework for illustrating the importance of proteolytic systems in plant defense, characteristics of non-proteasome proteases and the 26S proteasome are summarized. The involvement of caspase-like proteases, saspases, apoplasmic proteases, and the 26S proteasome in pathogen defense suggests that plant proteolytic systems are essential for defense and further clarity on the roles of plant proteases in defense is challenging but fundamentally important to understand plant-microbe interactions.

Keywords : defense, pathogen, plant proteases, proteasomes

Complex proteolytic systems in plants lead to the digestion of proteins into small peptides or amino acids (van der Hoorn, 2008). These systems perform the housekeeping function responsible for the removal of denatured proteins as well as for the adjustment of the stoichiometry of holoenzyme subunits (Buchanan et al., 2002). At least 723 and 955 putative protease genes have been identified within the genome of *Arabidopsis* and *Populus*, respectively (Garcia-Lorenzo et al., 2006). More and more additional proteases have been identified by *in silico* analysis, and in the review of van der Hoorn (2008), the number of protease genes in *Arabidopsis* genome increase to 826 from 488 (van der Hoorn and Jones, 2004).

The proteolytic enzymes are commonly termed proteases, proteinases, or peptidases. In this review, the term “proteases” is chosen. The proteolytic systems in plants include (i) proteases and (ii) proteasomes. Proteases are divided into five classes based on the amino acid determinants of

their catalytic site or their required metal cofactors, such as serine protease (EC 3.4.21), cysteine protease (EC 3.4.22), aspartic endopeptidase (EC 3.4.23), metalloprotease (EC 3.4.24), and threonine protease (EC 3.4.25) (Table 1) (Barrett et al., 2004). The catalytic classes are subdivided into Clans, and the Clans are further subdivided into Families based on the evolutionary relationship. An extremely useful updated site for classification and information on all known proteases can be found in the MEROPS database (<http://merops.sanger.ac.kr>) (Rawlings and Barrett, 1999; Rawlings et al., 2006).

In addition to the primary role of plant proteolytic systems in degrading nonfunctional proteins, the systems have been shown to be involved in various processes in response to developmental and environmental cues (Dreher and Callis, 2007; van der Hoorn, 2008). The high level of substrate-specificity and the tight regulation of temporal and spatial enzymatic activity imply that plant proteolytic systems are key regulators for cellular metabolism, such as development, cell cycle, cell growth and defense (van der Hoorn, 2008). Interestingly, studies have revealed that various proteolytic systems actively participate in the plant defense against pathogens (Table 2) (Dreher and Callis, 2007; van der Hoorn and Jones, 2004). The effects of proteases can be seen at different levels including: i) pathogen recognition, ii) the induction of defense responses, iii) signals leading to the release of positive regulators, iv) the degradation of negative regulators, or v) the execution of defense responses (van der Hoorn and Jones, 2004).

In this review, we summarize recent advances in the functional analysis of plant non-proteasomal proteases and the 26S proteasome playing important roles in plant-microbe interactions and defense. Each of these sets of proteolytic systems can be found at different sets of sub-cellular locations (Fig. 1). In addition to the 26S proteasome, several non-proteasomal proteases are chosen for the discussion of the role of proteases in defense, including caspase-like proteases in vacuole and saspases in cytosol as well as cysteine proteases, aspartic proteases and the matrixins in apoplasts.

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Table 1. Classification of proteases based on their catalytic funtions

Catalytic type	Active site	Synonyms	Clans	Characteristics
Aspartic proteases	Aspartate	Acid protease Aspartate protease	AA, AB, AC, AD, AF	All endopeptidases, e.g., pepsin
Cysteine proteases	Cysteine	Thiol proteinase, Sulfhydryl protease	CA, CD, CE, CF, CH, 'CX'	e.g., Papain family
Metallo-proteases	Metal cofactors	Metallopeptidase	MA, MC, MD, ME, MD, MJ, ML, MM, M11, MF, MG, MH, MN	Divalent metal cations, e.g. Zn, Co, Mn, Ni or Cu, mostly Zn-dependent
Serine proteases	Serine		PA(S), SB, SC, SE, SF, SH, SK, SM, SN	
Threonine protease	Threonine			Prototype members are the proteasome catalytic subunits

Roles of Plant Non-proteasomal Proteases in Pathogen Defense

Plant proteolytic systems are consisted of proteases and proteasomes. Proteasomes found in cytosol and in nucleus are multiunit complex, therefore, in this review, we summarizes the non-proteasomal proteases first. Caspase-like proteases and saspases are chosen to help explain the role of proteases in hypersensitive response (HR) to incompatible microbial pathogen attack. We also review the functions of extracellular secreted proteases in pathogen defense. The extracellular space is the interface of host-pathogen interaction and considered to be the molecular battlefield between plants and phytopathogens. The secreted extracellular proteases are the key players of plant defense at apoplasts and the summarized apoplastic proteases include i) papain-like cysteine proteases, ii) aspartic proteases, and iii) matrixins.

Caspase-like proteases implicated in plant defense (Clan CD). Caspases are the principal players for apoptosis in animal cells (Grütter, 2000). In animals, programmed cell death (PCD) can be classified into two categories, caspase-dependent or caspase-independent (van Doorn and Woltering, 2004). The key components of apoptotic cell death in animals are the caspases, a family of cysteine proteases which exist as dormant proenzymes in the cytosols of most cells. During apoptosis, a battery of caspases become activated through proteolytic processing at internal aspartic acid residues. The action of the active caspases on their substrates causes apoptotic morphological changes and eventually cell death.

Although caspase activities have been reported in plants for over 10 years, plants do not have orthologous sequences to animal caspase genes in their genome (Bonneau et al., 2008). Two families of caspase-like proteases are identified as paracaspases and metacaspases by *in silico* genome

Fig. 1. Subcellular localizations of the proteases and proteasomes involved in pathogen defense.

analysis (Uren et al., 2000). Paracaspases and caspases are present in animals, whereas metacaspases (MCPs) are found in plants, fungi, and protozoa. Plant metacaspases (family C14) are classified into type I and II, based on sequence and structural similarities. In the *Arabidopsis* genome, nine MCPs have been identified, two of which are upregulated by bacterial pathogens (Watanabe and Lam, 2004). In tomato, a metacaspase gene was upregulated following the infection of *Botrytis cinerea* (Hoeberichts et al., 2003). Single knock-out mutants of *Arabidopsis* metacaspases 2-6 were slightly but significantly less susceptible than wild-type to *B. cinerea* and *B. tulipae*, but not to *B. elliptica* (Van Baarlen et al., 2007). Plant metacaspases were suspected to have caspase-like activities (Madeo et al., 2002), however, they do not cleave caspase substrates (He et al., 2007; Watanabe and Lam, 2005). More studies are

Table 2. Pathogen-responsive proteases referred in this study.

Divisions ^a	Groups of proteases	Catalytic types	Subclasses	Names of genes	Plant species	Pathogens or elicitors	References
Caspase-like proteases		Cysteine proteases	Meta-caspases	<i>AtMCP1b</i> , <i>AtMCP1c</i> , <i>AtMCP2e</i>	<i>A. thaliana</i>	<i>Pseudomonas infestans</i> , <i>P. syringae</i> pv phaseolicola, <i>P. syringae</i> pv tomato DC3000	Wanatabe and Lam (2004) Sanmartín et al. (2005)
				<i>LeMCA1</i>	<i>S. lycopersicum</i>	<i>Botrytis cinerea</i>	Hoeberichts et al. (2003)
			Vacuolar processing enzymes (VPE)	<i>VPEγ</i>	<i>A. thaliana</i>	<i>P. syringae</i> , <i>B. cinerea</i> , and turnip mosaic virus	Rojo et al. (2004)
	Saspases	Serine proteases		<i>NtVPE-1a</i> , <i>NtVPE-1b</i> , <i>NtVPE-2</i> , <i>NtVPE-3</i>	<i>N. benthamiana</i>	Tobacco mosaic virus	Hatsugai et al. (2004)
				<i>SAS-1</i> , <i>SAS-2</i>	<i>A. sativa</i>	Victorin from <i>Cochliobolus victoriae</i>	Navarre and Wolpert (1999), Coffeen and Wolpert (2004)
Apoplastic proteases	Papain-like proteases	Cysteine proteases		<i>Rcr3</i> <i>PIP1</i>	<i>S. lycopersicum</i>	<i>Cladosporium fulvum</i> <i>P. infestans</i>	Krüger et al. (2002) Tian et al. (2007)
				<i>NbCYP1</i> , <i>NbCYP2</i>	<i>N. benthamiana</i>	<i>Colletotrichum destructivum</i>	Hao et al. (2006)
				<i>NtCath</i>	<i>N. benthamiana</i>	<i>Erwinia carotovora</i>	Gilroy et al. (2007)
	Aspartic proteases	Aspartic proteases		<i>StCath</i>	<i>S. tuberosum</i>	<i>P. infestans</i>	Avrova et al. (1999, 2004)
				<i>CDR1</i>	<i>A. thaliana</i>	<i>P. syringae</i>	Xia et al. (2004)
				<i>GmMMP2</i>	<i>G. max</i>	<i>Phytophthora sojae</i> or <i>P. syringae</i> pv glycinae	Liu et al. (2001)
Proteasomes	Matrixins	Metalloproteases		<i>MtMMP1</i>	<i>M. truncatula</i>	<i>Sinorhizobium meliloti</i>	Combier et al. (2007)
				β 1-tcl 7, α 3, α 6	<i>N. tabacum</i>	Cryptogein secreted by <i>Phytophthora cryptogea</i>	Petitot et al. (1997), Dahan et al. (2001)

^aDivisions mean the primary divided protease groups used in this review.

needed to elucidate the roles of plant metacaspases in defense, especially in regulating HR.

The plant vacuolar processing enzymes (VPE in family C13) are cysteine proteases, which were unexpectedly proven to have caspase-1 like activity and required for the HR in response to tobacco mosaic virus (TMV) (Hatsugai et al., 2004). Specifically, the mRNA and protein levels of four VPE cDNAs isolated from *Nicotiana benthamiana* were rapidly and transiently increased during early stages of HR in the TMV-infected tobacco leaves (Hatsugai et al., 2004). *VPE*-silenced *N. benthamiana* showed no visible lesions by TMV inoculation. The transcription of one of these genes (*VPE γ*) is induced by the infection with *Pseudomonas syringae*, *B. cinerea*, and turnip mosaic virus (Rojo et al., 2004). Knockout of *VPE γ* in *Arabidopsis* results in increased susceptibility to these pathogens, suggesting that VPEs contribute not only to resistance to

incompatible pathogens but also to the plant basal defense to compatible pathogens (Rojo et al., 2004). A fungal toxin fumonisin B1 induced cell death of *Arabidopsis*, however, in *Arabidopsis* *VPE*-null mutants lacking all four *VPE* genes, the features of cell death induced by fumonisin B1 were completely abolished (Kuroyanagi, 2005). No structural similarity between VPEs and caspases exists but the caspase-1 activity of VPE suggests that plants have evolved a novel cellular suicide mechanism using VPE and the cellular vacuole.

Saspase, a serine protease with caspase-like activity implicated in plant defense (Clan SB). The sensitive *Avena sativa* leaves treated with the victorin toxin from necrotrophic fungus *Cochliobolus victoriae* show symptoms of PCD (Navarre and Wolpert, 1999). Two proteases involved in the victorin-induced PCD signaling cascade

were purified and characterized (Coffeen and Wolpert, 2004). These proteases have caspase activity and belong to the family of plant subtilisin-like serine proteases (Bonneau et al., 2008; Sanmartín et al., 2005). Interestingly, these proteases have distinct substrate specificities compared to all other known subtilisin-like serine proteases. Saspase was coined because of their aspartate specificity (aspase) and active-site serine residue (S) of those proteases. Saspases are constitutively expressed in cells in an active form and appear to function in the apoplasts. A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* suppresses host cell death, and may target plant saspases to mediate this activity (Tian et al., 2004). A serine protease (Kunitz trypsin) inhibitor is triggered by phytopathogens and fumonisin B1 in *Arabidopsis*, and it antagonizes PCD in plant-pathogen interactions (Li et al., 2008). Although the mode of inhibition strongly suggests that the target proteases are saspases, the mode of action of cell death modulation and the target protease(s) have yet to be elucidated (Li et al., 2008).

Apoplastic proteases implicated in plant defense. Plant apoplasts are the interface between the plant and the interacting microenvironments. Plant cells secrete constitutively expressed proteins and inducible proteins involved in defense into apoplasts. Apoplasts interact with biotic and abiotic stresses, handle pathogen attacks, and effectively modulate the cellular metabolism (Agrios, 2005). Apoplasts provide a crucial defense against microbial pathogen attacks, and therefore can be described as a molecular battlefield for surveillance, assault, defense, and counter-defense (Lee et al., 2006). More than 50% of all proteases are predicted to be secreted into the apoplasts or into vacuoles as determined by our *in silico* tomato cDNA analysis (unpublished data). Therefore, clarifying the role of apoplastic proteases in pathogen defense will be a key step in understanding the plant-microbe interactions at the molecular battlefield.

Apoplastic papain-like cysteine proteases implicated in plant defense (ClanCA, C1A family). Papain is a small cysteine protease found in the latex of papaya trees, which has broad substrate specificity and plays an important role in defense (El Moussaoui et al., 2001). Multigenes of papain-like cysteine proteases (PLCPs), which share amino acid sequence homology and order of catalytic residues with papain, have been characterized in many plant species and known to have important roles in plant defense against microbial pathogens (Shabab et al., 2008). PLCPs, usually between 23-30 kDa, contain a cysteine residue at the catalytic site capable of cleaving peptide bonds in target proteins (Shindo and van der Hoorn, 2008). PLCPs have been grouped in the family C1, which has been further

subdivided into: i) apoplastic PLCPs (subfamily C1A) and ii) cytoplasmic PLCPs (subfamily C1B) in the MEROPS database. Many of the apoplastic PLCPs are induced by pathogen attack, and are part of a defense layer in the apoplastic battlefield.

The tomato apoplasts contain seven PLCPs classified into four different subfamilies (Shabab et al., 2008). Among the PLCPs, transcripts of *Rcr3* (Required for *Cladosporium* resistance-3) and *Pip1* (*Phytophthora* inhibited protease-1) are up-regulated upon pathogen attack, and accumulate in the apoplasts (Krüger et al., 2002; Tian et al., 2007). Treatment with benzothiazole, which triggers the salicylic acid-regulated defense response, induces only the transcripts of *Rcr3* and *PIP1*, suggesting the regulation of the genes by salicylic acid (Shahab et al., 2008). *Rcr3* is required for the function of the tomato Cf-2 receptor-like protein against the fungal pathogen *Cladosporium fulvum* carrying the *Avr2* avirulence gene (Krüger et al., 2002). However, the secreted *Avr2*, a cysteine-rich protein, binds and inhibits *Rcr3*, suggesting that *Rcr3* is rather a virulence target of *Avr2* that is guarded by the Cf-2 resistance protein to monitor pathogen entry (Jones and Dangl, 2006; Luderer et al., 2002; Rooney et al., 2005). *PIP1* is a pathogenesis-related protein closely related to *Rcr3*. *PIP1* is inhibited by *Epic2B*, a cystatin-like protease inhibitor secreted during infection by *P. infestans* (Tian et al., 2007). The *PIP1* and *Rcr3* genes map to the same locus of the tomato genome (Shindo and van der Hoorn, 2008), however, the higher accumulation of *PIP1* protein levels when compared to *Rcr3* indicates that *PIP1* might be the real virulence target of *Avr2* while *Rcr3* act as a decoy for *Avr2* perception in plants carrying the Cf-2 resistance gene (Jones and Dangl, 2006; Shabab et al., 2008). However, still the mode of how *Avr2* and *Epic2B* specifically inhibit PLCPs and of how Cf-2 recognizes the *Rcr3*-*Avr2* complex remains unclear (Shindo and van der Hoorn, 2008).

In *N. benthamiana*, two PLCPs, *NbCYP1* and *NbCYP2*, are differentially expressed during the susceptible response to *Colletotrichum destructivum* but not the resistant response to *P. syringae* pv. tomato (Hao et al., 2006). Virus-induced gene silencing (VIGS) of the two genes indicate that these PLCPs are not involved in the HR to *P. syringae* pv. tomato, but they are involved in limiting the host susceptibility to *C. destructivum* (Hao et al., 2006). *N. benthamiana* cathepsin B is an apoplastic PLCP, activated upon secretion in the absence of pathogen challenge, and required for disease resistance (Gilroy et al., 2007). VIGS of cathepsin B prevented PCD and compromised disease resistance induced by *Erwinia amylovora* and *P. syringae* pv. tomato DC3000, two non-host bacterial pathogens to tobacco (Gilroy et al., 2007). In *Solanum tuberosum*, *StCath*, a PLCP is also known as rapidly induced during R

gene-mediated potato interactions with *P. infestans* (Avrova et al., 1999; 2004).

Plant cystatins, cysteine protease inhibitors, show strong papain-inhibitory activities (Li et al., 1996). *AtCys1* (*Arabidopsis* cystatin-1) is strongly induced by wounding, challenge with avirulent pathogens, or by nitric oxide (Belenghi et al., 2003). The overexpression of *AtCYS1* blocks cell death caused by the infection of avirulent *P. syringae* (Belenghi et al., 2003). The inhibition of the PLCP enzymatic activity and of HR cell death by cystatins indicates that complex interactions between PLCPs and cystatins in apoplasts regulate the induction of HR and the spread of pathogens.

Apoplastic aspartic protease CDR1 implicated in plant defense (Clan AA, Family A1). The gene product of *A. thaliana* constitutive disease resistance 1 (CDR1) is an apoplastic aspartic protease and is a member of a larger group of the atypical aspartic protease (Simões et al., 2007). The overexpression of CDR1 causes dwarfing and resistance to virulent *P. syringae*, and the suppression of CDR1 results in compromised resistance to avirulent *P. syringae* and in increased susceptibility to virulent strains compared to the wild type. The role of CDR1 in defense is proposed to mediate the generation of an endogenous peptide signal involved in the activation of inducible resistance mechanisms (Xia et al., 2004).

Matrixins (or matrix metalloendopeptidases) implicated in plant defense (Clan MA(M), family M10A). The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells and is composed of structural proteins, specialized proteins and proteoglycans (Roberts, 1994). Matrixins are the proteases degrading and remodeling the structural proteins within the ECM (Barett et al., 2004). Matrixins are synthesized as prepro-enzymes and contain a signal peptide targeting them to the apoplast (Nagase and Woessner, 1999). The pro-matrixins are most likely activated proteolytically by certain groups of proteases *in vivo* as well as by mercurial compounds and reactive oxygen *in vitro* (Nagase and Woessner, 1999). Studies of matrixins in vertebrates revealed their role in development, embryogenesis, organ morphogenesis, and wound healing, as well as in pathological processes such as cancer and arthritis (Barett et al., 2004). The studies with animal matrixins suggest that plant matrixins may be essential regulators of growth and development and even have an important role for plant defense and PCD.

Several matrixins were isolated from plants, including soybean (Graham et al., 1991; Liu et al., 2001; McGeehan et al., 1992; Pak et al., 1997), *Arabidopsis* (Maidment et al., 1999), cucumber (Golldack et al., 2002), and alfalfa

(Combiér et al., 2007). Matrixins hold the HEX2HX2G-X2HS zinc-binding catalytic motif and are sensitive to EDTA (McGeehan et al., 1992). The first isolated matrixin from higher plants was SMEP-1 (soybean metallo-endoproteinase-1) from soybean (Graham et al., 1991), and is localized to the apoplasts of leaves. SMEP-1 showed both structural and biochemical similarities with vertebrate matrixins (McGeehan et al., 1992). The M10A family of vertebrate matrixins includes a number of proteases, such as collagenases, gelatinases, stromalysins, and matrilysin, etc. (Barrett et al., 2004).

GmMMP2, the second matrixin from soybean was identified as a gene which is transcriptionally upregulated during compatible and incompatible interactions with the oomycete pathogen *Phytophthora sojae* or the bacterial pathogen *P. syringae* pv *glycinae*, suggesting that it may be involved in a novel defense mechanism (Liu et al., 2001). Interestingly, *GmMMP2* is not induced by the classical pathogen-induced signaling molecules such as salicylic acid and jasmonic acid, and furthermore does not correlate with the oxidative burst leading to HR (Liu et al., 2001). *MtMMPL1*, a *Medicago truncatula* matrixin represents a novel and specific marker for root and nodule infection by *Sinorhizobium meliloti* (Combiér et al., 2007). The loss-of-function study employing an RNA interference approach to decrease *MtMMPL1* expression revealed an accumulation of *Rhizobia* within infection threads, often accompanying significantly enlarged diameter of the threads. The overexpression of *MtMMPL1* in a gain-of-function study resulted in numerous abortive infections and an overall decrease in the number of nodules (Combiér et al., 2007). Although the roles of plant M10A matrixins are not yet fully understood, plant matrixins seem to be involved in a novel defense mechanism and may even be involved in plant PCD.

Roles of the Plant 26S Proteasome in Pathogen Defense

Most cytoplasmic and nuclear proteins are degraded in a ubiquitin (Ub) and 26S proteasome-mediated manner (Delauré et al., 2008). The non-lysosomal intracellular protein degradation mediated by this pathway is essential for the regulation of various cellular processes, such as development, cell cycle, cell growth, and transcription (Jessenberger and Jentsch 2002). The 76-amino acid protein Ub is attached to proteins destined for degradation, and then the resulting Ub-protein conjugates are tagged for catalization by the 26S proteasome (Smalle and Vierstara, 2004). The 26S proteasome contains 31 principal subunits and is composed of a 20S core proteasome and a 19S regulatory particle (Beers et al., 2000). Association of the 20S core proteasome with the multisubunit 19S regulatory particle creates the ATP-dependent, 2,100 kDa, 26S proteasome

(Samille and Vierstra, 2004). Recently, evidence is heavily accumulating which suggests that the ubiquitin/proteasome pathway may control a range of cellular signaling processes in plants, such as those mediated by hormones, light and sucrose, as well as development and response to pathogens (Ellis et al., 2002; Smalle and Vierstara, 2004). The ubiquitin cascade is operated sequentially in a multi-step fashion by a multi-enzymatic system, consisting of ubiquitin-activating (E1), -conjugating (E2), and -ligating (E3) enzymes (Delauré et al., 2008; Glickman and Ciechanover, 2002). E3 ubiquitin ligases mediate the final transfer of ubiquitin to the target proteins, and therefore, they represent an important portion of the degradation process. Increasing numbers of studies illustrate the involvement of E3 ubiquitin ligases in plant defense (Kuprea and Smalle, 2008). More than 1,300 genes encoding for a E3 ubiquitin ligase motif are present in the *Arabidopsis* genome (Kraft et al., 2005). The E3 ubiquitin ligases can be classified in two major groups: those containing a HECT (for homology to E6-associated protein C-terminus) domain and those with a RING (really interesting new gene) or RING-like (called U-box) domain. The *in silico* analyses on the expression of all annotated E3 ubiquitin ligase components reveal that biotic stress induces up-regulation and down-regulation of up to 548 and 382 E3 ubiquitin ligase components, respectively (Delauré et al., 2008). E3 ubiquitin ligases and the related protein breakdown have been shown to play an important role in the signal transduction pathways leading to disease resistance (Delauré et al., 2008; Devoto et al., 2003; Dreher and Callis 2007). E3 ubiquitin ligases are involved in plant defenses by controlled proteolysis in i) 'gene-for-gene' disease resistance, ii) early defense response reactions and iii) late induced defense responses (Delauré et al., 2008; Read Dreher and Callis, 2007, and Delauré et al., 2008 to get more detailed information).

Proteasomes are known to be involved in the plant defense in response to pathogen attacks (Dahan et al., 2001; Groll et al., 2008; Kurepa et al., 2008). Specifically, competence development was greatly suppressed by specific inhibitors of proteasome activity in cucumber hypocotyls (Becker et al., 2000). Treatment with cryptogein, a proteinaceous elicitor of plant defense reactions induced the upregulation of only the genes encoding b1-tcI 7, α 3 and α 6, 20S proteasome subunits in tobacco (Petitot et al., 1997; Dahan et al., 2001). The defense-induced (din) 20S subunits are induced only in the conditions of the induction of systemic acquired resistance and of the production of reactive oxygen species (Suty et al., 2003). Production of reactive oxygen species in tobacco plants treated with cryptogein is mediated by the NADPH oxidase and triggers the accumulation of β 1 din 20S subunits (Lequeu et al., 2005). Gain- or loss- of functional studies for the β 1 din

20S subunits demonstrate that a proteasome composed of a β 1 din 20S subunit acts as a negative regulator of NADPH oxidase during the elicitation of plant defense reactions, and in this manner contributes to the regulation of ROS generation during the pathogen attack (Lequeu et al., 2005).

Conclusions and Perspectives

There have been massive accumulations of exciting reports on the crucial involvement of plant proteolytic systems on pathogen defense. The study of the proteolytic systems on pathogen defense will be very challenging due to the huge numbers of proteases, their redundancy, cellular locations, activation mechanism, and substrates variances. However, considering the roles of proteolytic systems in perception, signaling, and execution leading to plant defense (van der Hoorn and Jones, 2004), elucidation of the functions of these diverse proteolytic systems in defense will be one of the most important studies for understanding the interaction between plants and pathogens. In the next decades, further advances will provide astonishing discoveries on the roles of plant proteolytic systems in defense by combining the newly advancing functional genomics and proteomics with the studies determining the substrates for the candidate proteases or proteasomes.

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