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Chapter Title	Programmed Cell Death in Plants	
Chapter SubTitle		
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Summary	Throughout the life cycle of plants, programmed cell death (PCD) is involved in a wide range of developmental processes and responses against abiotic or biotic stresses. PCD is an active form of cellular suicide controlled by a network of genes. Such phenomenon is associated with recovery of cellular

compounds and sustaining plant life. Basic morphological and biochemical features of PCD are believed to be conserved in both plants and animals. Nevertheless, recent studies demonstrate an involvement of organelles such as vacuole and chloroplast in plant cell death regulation, indicating that plants evolved own cell death machinery. Reactive oxygen species (ROS) generated by biotic and abiotic stresses act as a signal that induces plant PCD. This article describes some of the fundamental characteristics of plant PCD and raises points that may lead to a better understanding and novel strategies for plant molecular breeding.

Keywords (separated by '-')	Aerenchyma - apoptosis - mitochondria - oxidative stress - programmed cell death - reactive oxygen species

Chapter 17

Programmed Cell Death in Plants

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A. Pareek et al. (eds.), Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation, pp.000–000.
DOI 10.1007/978-90-481-3112-9_17, © Springer Science+Business Media B.V. 2009.

Summary

Throughout the life cycle of plants, programmed cell death (PCD) is involved in a wide range of developmental processes and responses against abiotic or biotic stresses. PCD is an active form of cellular suicide controlled by a network of genes. Such phenomenon is associated with recovery of cellular compounds and sustaining plant life. Basic morphological and biochemical features of PCD are believed to be conserved in both plants and animals. Nevertheless, recent studies demonstrate an involvement of organelles such as vacuole and chloroplast in plant cell death regulation, indicating that plants evolved own cell death machinery. Reactive oxygen species (ROS) generated by biotic and abiotic stresses act as a signal that induces plant PCD. This article describes some of the fundamental characteristics of plant PCD and raises points that may lead to a better understanding and novel strategies for plant molecular breeding.

Keywords: Aerenchyma • apoptosis • mitochondria • oxidative stress • programmed cell death • reactive oxygen species

I Introduction

In multicellular organisms, specific cells commit suicide to achieve and maintain homeostasis by specifically ordered metabolic changes during normal development, environmental stress, or pathogen attack. This functionally conserved and gene-directed cell death process is known as programmed cell death (PCD). The phenomenon depends on active participation of the dying cells, and could be regulated by genetically controlled, well-orchestrated cell suicide machinery. The common process in such instances comprise one or more phenotypes such as cytoplasmic shrinkage, membrane blebbing, loss of cell-to-cell contact, DNA fragmentation and disassembly of the nuclei (Lam et al. 2001; Lam 2004). Today, ample evidence can be presented to support that cell death during plant development and environmental challenges involves PCD (Fig. 1).

PCD occurs in numerous vegetative as well as reproductive phases of plant development, including senescence of leaves (Gan and Amasino 1997), development of tracheary elements (Zhang et al. 2002), timely death of petals after fertilization (Havel and Durzan 1996), post-embryonic decay of aleurone layers (Wang et al. 1996b), root cap development (Moller and McPherson 1998), somatic as well as zygotic embryogenesis (Giuliani et al. 2002) and sex determination (De Long et al. 1993). A number of abiotic stresses such as salinity, extreme temperatures, excess light and UV radiation lead to production of ROS (Dhariwal et al. 1998). Reactive oxygen species (ROS) generated by biotic and abiotic stimuli act as molecules that function at the early stage of signal transduction, stress adaptation and PCD. Signaling responses of ROS include activation of MAPK related to hypersensitive response – HR (Hancock et al. 2002). Exogenously supplied ROS, such as H_2O_2, also induces cell death in soybean (Levine et al. 1994), tobacco (Houot et al. 2001), and *Arabidopsis* (Tiwari et al. 2002), which includes cell shrinkage, DNA fragmentation and chromatin condensation.

The cell death process can be divided into three phases: an induction phase, the nature of which depends on the specific death-inducing signals; an effector phase, during which the cells commit to die; and a degradation phase, where the biochemical and morphological features of cell collapse can be observed (Martins and Earnshaw 1997).

Abbreviations: CaM – calmodulin; CERK – ceramide kinase; ER – endoplasmic reticulum; HR – hypersensitive response; LRR – leucine-rich repeat; MAPKs – mitogen activated protein kinases; MTP – mitochondrial-permeability transition pore; NBS – nucleotide binding site; PCD – programmed cell death; PK – protein kinase; PS – phosphatidyl serine; ROS – reactive oxygen species; SERCA – sarco endoplasmic reticulum Ca^{2+} ATPase; TM – transmembrane-domain; TMV – tobacco mosaic virus; TNF – tumour necrosis factor; VPE – vacuolar processing enzyme

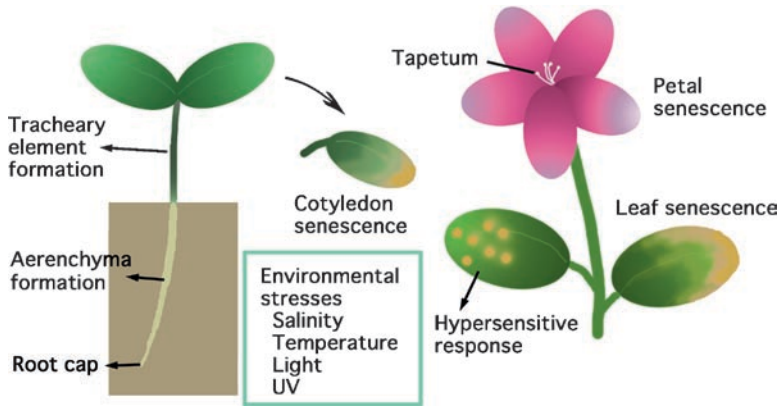

Fig. 1. PCD occurs in plant life cycle. PCD is involved in many phases through vegetative and reproductive development and response to environmental stresses.

In plants, some PCD resembles either a common form seen in animals called apoptosis or it resembles a morphologically distinct form of cell death (Pennel and Lamb 1997). However, distinctive characteristics of plant cells including the existence of a cell wall imply that there are dissimilarities in the execution of PCD. The cell wall precludes phagocytosis, establishing a different mechanism for corpse management. Similarly, the vacuole can be transformed into a hydrolytic compartment with hydrolases and toxin profiles (Paris et al. 1996) that degrade the components of dying cell after collapse of the vacuole.

II Anatomy of Cell Death

Plant cells are characterized by the presence of cell wall. The cell wall may or may not be degraded along with the protoplast, depending on the type of PCD (Jones 2001). During tracheary element differentiation, the primary wall and a rigid secondary wall are required for cell function and are not hydrolyzed, except for portion of the primary wall between the adjacent tracheary elements that is degraded to from perforations (Nakashima et al. 2000). In most other forms of developmental PCD, collapsed primary cell walls are left behind, whereas nutrients from dismantled protoplast are recycled (He and Kermode 2003). When the HR is induced by pathogen invasion, the protoplast dies, leaving collapsed or crushed primary cell wall behind (Mittler and Lam 1997). Lysogenic aerenchyma formation involves death and often complete lysis of cells, with the disappearance of all cell components, including the cytoplasm and cell walls (Kozela and Regan 2003). Because plants do not have macrophages, dying cells must degrade their materials by themselves. In the case of rice seminal roots, the gas-space caused by lysogenous cell death expands radially, leaving behind structures derived from cell wall (Fig. 2). The first cell to collapse is located at a specific cell position in mid cortex (Kawai et al. 1998), and such lysigenous aerenchyma formation is regulated by ethylene (He et al. 1996). Furthermore, stresses such as NaCl treatment affect the cortical cell death and cell proliferation in roots of rice (Samarajeewa et al. 1999).

One of the common features of animal cells undergoing apoptosis is development of membrane asymmetry. Exposure of phosphatidyl serine (PS) on the outer and inner surface of plasma membrane triggers such phenomenon. Externalized PS appears to serve as an important signal for recognition and elimination of apoptotic cells by macrophages (Ceccatelli et al. 2004). Similar membrane asymmetry has also been observed in plants during PCD. In tobacco, changes in PS asymmetry, analyzed by measuring Annexin V bound to the cell membrane, were detected by a number of chemical agents (O'Brien et al. 1998). Similar findings were also reported in apple suspension cells under a low oxygen culture (Xu et al. 2004). However,

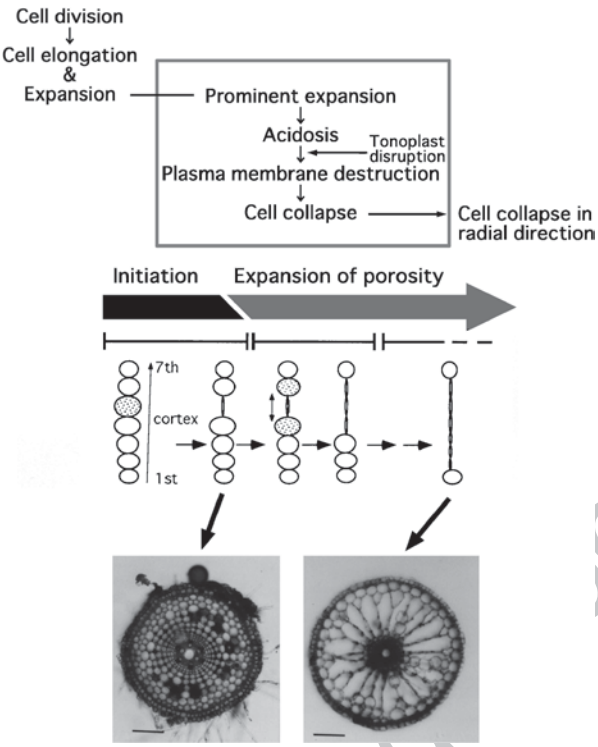

Fig. 2. Aerenchyma formation in rice roots follows a well designated cell fate. Lysigenous gas-space formation initiates at specific position of the mid cortex and expand toward basal portion of rice seminal root. In the neighborhood of the meristematic region, cell enlargement occurs. Cells in the mid cortex expand greatly, followed by acidification. Some cells in the mid cortex lose turgor pressure due to tonoplast disruption, followed by the loss of plasma-membrane integrity. Cells appear somewhat concave, lose contact with neighboring cells and collapse. Once cell collapse begins, neighboring cells die. The cavity then expands radially. Bar = 0.1 mm.

the physiological role of PS exposure in plants is still unknown.

III Biochemistry of Cell Death

Much of the evidence for the concept of apoptosis in plants is derived from the observation of DNA fragmentation in cells entering the cell death phase. In animals, the cleavage of DNA has been found to occur at the inter-nucleosomal sites resulting in DNA fragments of 180 bp (Walker and Sikorska 1994). This form of DNA fragmentation can be detected by molecular detection of DNA ladders (multimers of 180 bp) on agarose gels. DNA laddering has been observed in plant tissues responding to fungal infection or phytotoxin exposure (Ryerson and Heath 1996; Wang et al. 1996a), in senescing carpels (Orza´ez and Granell 1997), in hormone treated aleurone cells (Wang et al. 1996b), and in cells or tissues responding to abiotic stress treatments (Wang et al. 1996a). Using TUNEL assay, which detects PCD *in situ*, fragmented DNAs are detected in senescent leaves (Yen and Yang 1998), in tracheary element differentiation (Mittler and Lam 1995) and in senescent coleoptile (Kawai and Uchimiya 2000). Nuclear shrinkage is also presented as other apoptotic features in plant (Katsuhara and Kawasaki 1996; Orza´ez and Granell 1997).

Endonucleases responsible for plant cell death have been characterized. Ito and Fukuda (2000) identified ZEN1 as a key nuclease responsible for nuclear degradation during the terminal stages of tracheary element differentiation. ZEN1 is a Zn^{2+}-requiring nuclease and its activity is insulated in the vacuole.

In animal apoptosis, caspases, cytosolic family of cysteine proteases that specifically cleave adjacent to an aspartate residue, have pivotal

roles in execution of cell death (Thornberry et al. 1997). They are synthesized as inactive proenzymes and are activated by directed proteolytic removal of N-terminal peptide (Grutter 2000). In general, apoptotic cell death involves a sequence of caspase activation events in which initiator caspases such as Casp 8 and 9 activate downstream caspases (Casp 3, 6, 7) which in turn process a variety of target proteins eventually leading to the apoptotic phenotype (Woltering et al. 2002). Although the existence of caspase orthologs in plants is controversial, cysteine protease or caspase activity has been reported in plant systems undergoing PCD (Collazo et al. 2006). Uren et al. (2000) distinguished two families of caspase-like proteins, one from animals and slime mold and the other from plants, fungi, and protozoa. These are designated as paracaspases and metacaspases, respectively. The mcII-Pa protein, one of the metacaspase in *Picea abies* expressing in embryonic tissues is committed to PCD in embryogenesis (Suarez et al. 2004). Plant cell death can be suppressed using synthetic or natural caspase inhibitors. For example, VEIDase activity (equivalent to human caspase 6) was known as the main caspase-like activity in embryogenesis in *Picea abies* (Bozhkov et al. 2004). Expression of antiapoptotic baculovirus p35 gene, a caspase inhibitor in mammalian system, blocks PCD in tomato (Lincoln et al. 2002). Furthermore, caspase activities are detected in tobacco after invasion by pathogens (del Pozo and Lam 1998), in tomato after chemical-induced apoptosis (De Jong et al. 2000) and in *Arabidopsis* after treatment with nitric oxide (Clarke et al. 2000).

Recently, vacuolar processing enzyme (VPE) was identified as a plant caspase. VPE-deficient plants showed inhibited cell death in HR and in embryogenesis (Hara-Nishimura et al. 2005). Although VPE is not homologically related to the caspase family or metacaspase family, it shares similar enzymatic properties with caspase 1. Unlike animal apoptosis, plants might have evolved a cell death system that is, in some case, mediated by VPE.

IV Role of Vacuole

Cells destined to die are disposed off by the hydrolytic enzymes sequestered in the vacuoles (Jones 2001). The hydrolases sequestered in the vacuole are released when the vacuole collapses. This collapse is an irreversible step towards death which results in the immediate cessation of cytoplasmic streaming and requires a calcium flux (Jones 2000, 2001).

This execution process is based on the integration of various signals such as auxins, cytokinins, ethylene and elicitors (Jones 2001). In tracheary elements differentiation, auxin and cytokinins induce the de novo synthesis of vacuole sequestered nucleases and proteases, leading to complete degradation of cellular content leaving behind the extracellular matrix and the secondary cell wall built before death (Gunawardena et al. 2004). During the formation of lysigenous aerenchyma, induced by ethylene, the dead cells are removed and the cell wall hydrolases, such as cellulase are induced to fulfill the need to remove not only the protoplasm but the extracellular matrix as well, resulting in gas space formation (Schussler and Longstreth 2000). Vacuolar hydrolytic enzymes are released into the cytosol to attack various organelles, leading to cell death (Fukuda 2004). The caspase 1-like cysteine protease, VPE, in plant is also localized in the vacuole (Hara-Nishimura et al. 2005). VPE deficiency suppresses vacuolar collapse leading to cell death, suggesting that VPE functions as a key molecule in vacuolar collapse-triggered cell death.

V Role of Mitochondrion

Mitochondria are major sites of energy conversion and carbon metabolism in the cell. Mitochondria play a central role in integrating signals, regulator and adaptor molecules for regulation and execution of mammalian cell death. Mitochondria can trigger apoptosis from diverse stimuli through the opening of mitochondrial permeability transition pore (MTP), which allows release of the apoptosis-inducing factor and translocation of cytochrome *c* into the cytosol (Green and Reed 1998). In *Arabidopsis* cells, oxidative stress increases mitochondrial electron transport, resulting in amplification of H_2O_2 production, depletion of ATP and cell death. The increased generation of H_2O_2 also caused the opening of MTP and the release of cytochrome *c* from mitochondria (Tiwari et al. 2002). The release of cytochrome *c* and cell death was prevented by a serine/cysteine protease inhibitor. ROS-treated plant mitochondria

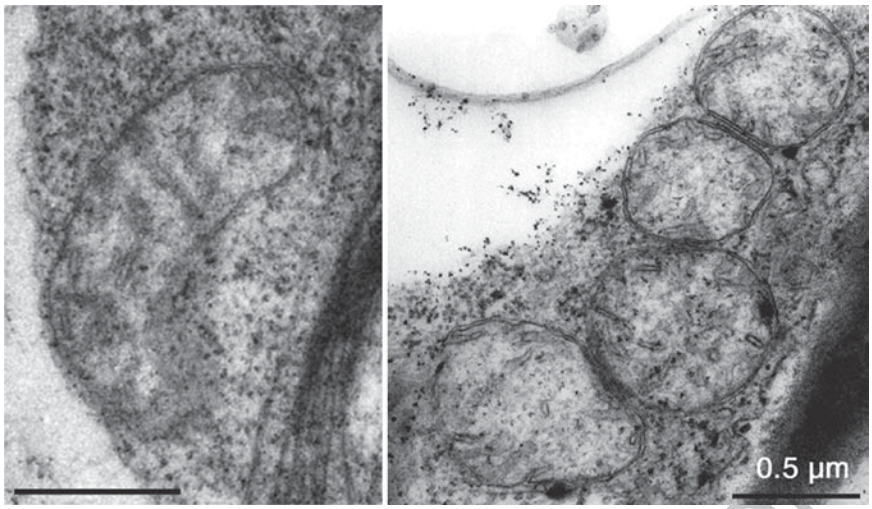

Fig. 3. Morphological changes in mitochondria during ROS-induced plant cell death. Mitochondria were observed by electron microscope at 0 (control, *left*) or 3 days (*right*) after cell death induction (Partially modified from Yoshinaga et al. 2005a).

showed morphological change from bacillus-like shape to a round shape (Fig. 3). Furthermore, the mitochondrial size decreased by half under ROS stress (Yoshinaga et al. 2005a, b). Mitochondrial fission proteins (Dnm1, Mdv1, Fis1) regulate cell death in animal and yeast cells by leading the mitochondrial fragmentation (Fannjiang et al. 2004; Karbowski et al. 2004). Such morphological changes suppress energy production of mitochondria and execute plant cell death.

In comparison to animal cells, plant mitochondrion has some unique components that alter mitochondrial functions towards PCD. One of these components is mitochondrial alternative oxidase (AOX) that functions as a part of an alternative electron pathway. This enzyme has been identified in *Arabidopsis* as an early induced gene in HR (Lancomme and Roby 1999). An over-expression of AOX in transgenic tobacco plants carrying the R gene resulted in reduced HR lesions following viral infection (Ordog et al. 2002). Furthermore, the treatment of tobacco cells with inhibitors of the cytochrome *c* pathway (Cys and antimycin A) was accompanied by a strong induction of the AOX capacity and the prevention of cell death (Vanlerberghe et al. 2002).

The host-selective toxin victorin, produced by *Cochliobolus victoriae* causes Victoria blight of oat. Victorin binds to P protein of the glycine decarboxylase (GD) complex localized in the mitochondrial matrix (Wolpert et al. 1994), inhibits GD activity, and induces apoptosis-like responses such as chromatin condensation and DNA laddering (Navarre and Wolpert 1995: Yao et al. 2001; Guo and Crawford 2005).

Furthermore, Kim et al. (2006) showed that virus-induced gene silencing (VIGS) of mitochondrial hexokinase caused necrotic lesion of leaves in *Nicotiana benthamiana*. These cells also showed nuclear condensation and DNA fragmentation, which are morphological markers of PCD. These findings suggest a pivotal role of mitochondria in the regulation of plant cell death.

VI Role of Chloroplast

Light requirement for PCD has often been associated with the production of ROS during photosynthesis (Martienssen 1997). Seo et al. (2000) demonstrated that the DS9 gene encoding FtsH protein in chloroplast is involved in the cell death regulation in tobacco mosaic virus (TMV)-mediated HR. Transgenic tobacco over-expressing DS9 stimulated HR cell death. In contrast, DS9 deficient plant displays less necrotic lesions. *Arabidopsis* mutant *psi2* (phytochrome signaling) showed light dependent super-induction of the pathogen-related protein PR-1a and developed spontaneous necrotic lesions in the absence of pathogen infection (Genoud et al. 1998). The PSI2

product negatively regulates photo-transduction pathways downstream of both phyA and phyB.

The lesion initiation 1 (*len1*) mutant of *Arabidopsis*, having the defective chloroplast chaperonin (Cpn60), developed lesions on its leaves in a light dependent manner. The *len1* leaves had a wrinkled irregular surface and displayed lesion formation when they were grown under short-day conditions. Under long-day conditions, the lesion formation was suppressed (Ishikawa et al. 2003). In addition, *lls1* (lethal leaf spot 1) mutant in maize is also characterized by the light-dependent formation of necrotic spots (Gray et al. 2002). The Lls1 gene encodes a protein possessing Rieske-type Fe-sulfur center domain. In *acd2 Arabidopsis* mutant, the photo-activation of the red chlorophyll catabolite triggers free radical production and subsequent cell death (Mach et al. 2001). These results clearly support the notion that light energy is used directly or indirectly to produce cell death mediators such as ROS or phototoxic chlorophyll intermediates, triggering the death.

VII Signals in Cell Death

Signals that initiate the process of cell death are passed on inside the cell through a number of cascades (Fig. 4). These signals, especially ROS signal is believed to be mediated through alterations in Ca^{2+}-fluxes, redox changes, ATP depletion, membrane vulnerability, ion leakage and disruptions to cellular function. For example, the altered NAD(P)H pool may confer the prevention of ROS-induced cell death (Hayashi et al. 2005).

The death receptors belong to the tumour necrosis factor (TNF) receptor super family or to the Fas receptors in animals and proteins encoded by R genes in plants (Kam 2000). The R genes are activated through specific interactions with avirulence proteins generated only by certain types of pathogens. The R genes encode several classes of proteins possessing domains of nucleotide binding sites (NBS), leucine-rich repeats (LRR), transmembrane domains (TM), and serine threonine protein kinases (PK). The majorityof these proteins have the NBS-LRR structure and are believed to be functionally confined to disease resistance. This class of R proteins may be further subdivided depending on the presence or absence of an N-terminal Toll/Il-1 receptor (TIR) domain. The NBS domain shows homology to regions found in the pro-apoptotic regulator Apaf-1 (Van der Biezen and Jones 1998). Apaf-1 and these proteins also share a similar structural organization. Thus, the common nucleotide binding (NBS) domain shared by these proteins links an effector domain (CARD in Apaf-1

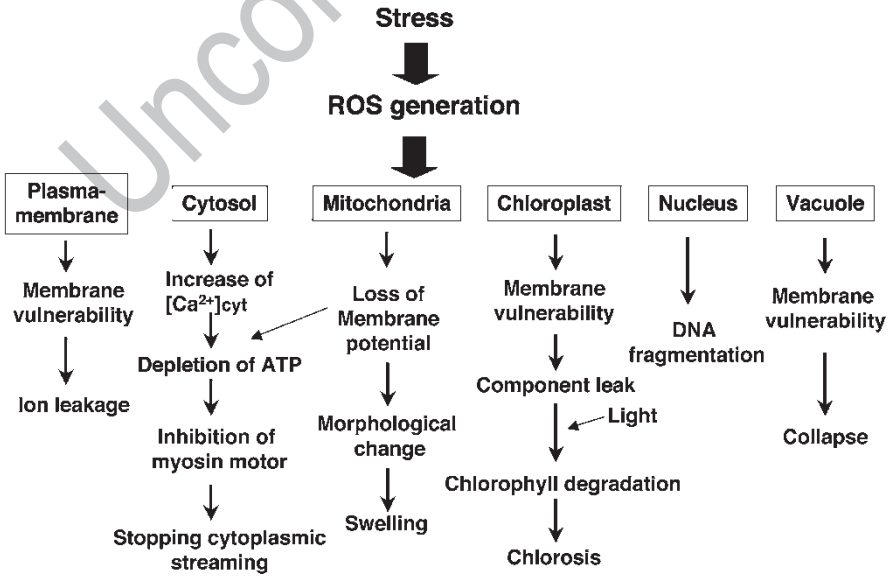

Fig. 4. Biological processes leading to oxidative stress-induced cell death. Abiotic and biotic stresses lead to ROS accumulation, which triggers orchestrated events in plant cells.

and TIR in these proteins) to a C-terminal domain likely to be involved in protein-protein interactions [WD domains in Apaf-1 and LRR domain in R proteins] and both are involved in cell death (Inohara et al. 1999).

As in the case of animals, following the receipt of a stimulus plant death receptors are activated, which in turn affect a number of signal pathways by protein phosphorylation, lipid-mediated signaling and a modification in ion fluxes. Mitogen-activated protein kinase (MAPK) cascades have become one of the most widely studied pathways of phosphorylation signaling related to PCD. Two *Arabidopsis* MAPKKs, AtMEK4 and AtMEK5, are functionally interchangeable with tobacco NtMEK2 in activating the downstream MAPKs. In the case of transient transformation experiments, performed in tobacco, the active forms of AtMEK4 and AtMEK5 activate endogenous tobacco SIPK and WIPK. These two MAPKKs, as well as tobacco NtMEK2 also activate two endogenous MAPKs, followed by the HR-like cell death (Ren et al. 2002). Oxidative stress-activated MAP triple kinase 1 (OMTK1) can specifically activate the downstream MAP kinase MMK3, which can also be activated by ethylene and elicitors, thus serving as a convergence point of the cell death network (Nakagami et al. 2004).

The activity of protein kinases is simultaneously regulated by cofactors and second messengers such as calcium. A MAPK phosphatase gene (NtMKP1), ortholog of *Arabidopsis* MKP1, was isolated as a candidate gene for a calmodulin (CaM)-binding protein from tobacco. In transgenic tobacco over-expressing NtMKP1, the wound-induced activation of SIPK, salicylic acid-induced MAPK and WIPK were inhibited. These results suggest that plant CaMs are involved in these stress-activated MAPK cascades via NtMKP1 (Yamakawa et al. 2004).

Sphingolipids are essential components of eukaryotic membranes that not only serve as modulators of extracellular interactions and cell surface receptors but also have critical functions as intracellular signaling messengers. The sphingolipid pathway generates three signaling metabolites known to function in intracellular signaling i.e., ceramide, sphingosine and sphingosine-1-phosphate, These metabolites play important roles in cell growth and differentiation (Hannun and Obeid 2002; Liang et al. 2003). Ceramide signaling pathway serves as a critical second-messenger system and has been studied in detail to understand apoptosis during degenerative and proliferative disease expressions in animal systems. The balance between the bioactive sphingolipid ceramide and its phosphorylated derivatives modulate PCD in animals as well as plants (Hannun and Obeid 2002). As second messengers, sphingolipids and sphingoid bases regulate cell behavior at many levels, including cell-to-cell communication, growth factor receptors, growth, differentiation and transformation (Ng and Hetherington 2001). The interplay between sphingolipid metabolite sphingosine-1-phosphate and heterotrimeric G-proteins represents an evolutionary conserved signal transduction mechanism in plants (Coursol et al. 2003). *Arabidopsis* ceramide kinase (CERK) mutant, called *acd5*, accumulated CERK substrates, and showed apoptosis-like phenotype (Liang et al. 2003). Acid tolerance response 1 (*Atr1*) mutants, tolerant to AAL toxin, are also resistant to H_2O_2-induced death, suggesting the involvement of ROS in sphingolipid metabolism for regulation of cell death (Gechev and Hille 2005).

VIII Cell Death Regulator

Bax, known as a mammalian proapoptotic protein, causes cell death when expressed in plants and yeast (Madeo et al. 1999; Baek et al. 2004; Yoshinaga et al. 2005a, b). Using such heterologous system, candidates of plant cell death regulators were isolated (Kawai-Yamada et al. 2005a, b). Bax inhibitor-1 (BI-1) is one such death suppressor that is conserved in metazoans and plants (Xu and Reed 1998; Lam et al. 2001; Chae et al. 2003; Hückelhoven 2004). Plant BI-1 genes isolated from rice (Kawai et al 1999), *Arabidopsis* (Kawai et al. 1999; Sanchez et al. 2000; Yu et al. 2002), tobacco (Bolduc and Brisson 2003), *Brassica* (Bolduc and Brisson 2003) and barley (Hückelhoven et al. 2001) have been intensively studied in yeast, plant and mammalian system. The BI-1 protein has six or seven transmembrane domains and is localized in the endoplasmic reticulum (ER) membrane (Xu and Reed 1998; Kawai-Yamada et al. 2001; Bolduc et al. 2003). Plant cells over-expressing AtBI-1 demonstrated cell death suppression in response to Bax-, salicylic acid-,

		Charge of replaced amino acid	Score for coiled-coil	Cell death suppression
AtBI-1	NSADKEEKKKKRRN	+	0. 88	+
AtBI28	NSADKEEEEEEEEN	–	0. 99	+
AtBI29	NSADKEEQQQQNNN	non	0. 84	+
AtBI30	NSADKEEIIIIGGN	non	0. 01	–
AtBI31	NSAKKKKKKKKRRN	+	0. 80	+
AtBI32	NSANNQQKKKKRRN	non	0. 72	+

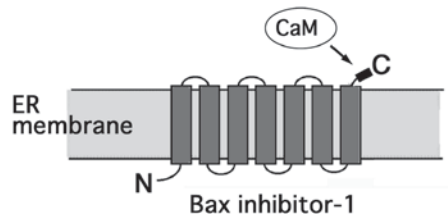

Fig. 5. The C-terminal region of AtBI-1 is essential for the cell death inhibition. The C-terminal 14 amino acids of AtBI (original) were replaced in mutants (AtBI-28-32). The score for the coiled-coil structure and the ability for cell death suppression are indicated. The AtBI30 mutant lacking coiled-coil structure failed to inhibit cell death, suggesting that the C-terminal region is essential for the inhibition of cell death through protein-protein interaction (Ihara-Ohori et al. 2007)

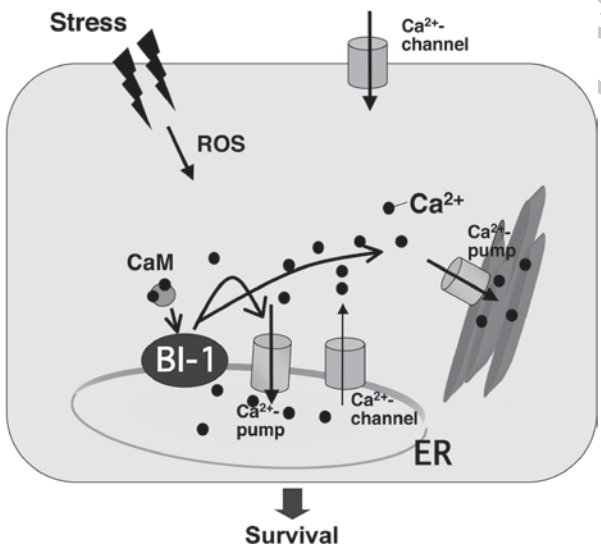

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Fig. 6. Proposed model of calcium flux and BI-1. BI-1 may regulate calcium flux at ER in response to stresses, leading to inhibition of cell death in plant cells. ROS stimulation caused by various stress conditions induces an increase of $[Ca^{2+}]_{cyt}$ through Ca^{2+}-channels. In contrast, the Ca^{2+}-pump uptake calcium into internal stores such as endoplasmic reticulum (ER) and golgi to prevent elevation of $[Ca^{2+}]_{cyt}$. Regulation of intracellular Ca^{2+} homeostasis is crucial for suppressing cell death. The CaM-binding BI-1 may regulate Ca^{2+} flux at ER. BI, Bax inhibitor-1, CaM, calmodulin

elicitor-, and H_2O_2-induced cell death (Kawai-Yamada et al. 2001; Matsumura et al. 2003; Kawai-Yamada et al. 2004). AtBI-1 may act down-stream of ROS generation (Kawai-Yamada et al. 2004). The C-terminal mutant of AtBI-1, lacking a coiled-coil structure, fails to inhibit cell death (Fig. 5). Recently, calmodulin was isolated as an interactant of C-terminal region of AtBI-1 (Ihara-Ohori et al. 2007). Calmodulin binding to AtBI-1 modulates calcium flux in plant cells. The AtBI-1 over-expressing or knock-down plants demonstrated an altered sensitivity against CPA (inhibitor of SERCA type Ca^{2+}-ATPases) and ion stresses, suggesting that AtBI-1 plays a role in ion homeostasis in case of plant cell death regulation (Fig. 6).

IX Concluding Remarks

Cell death in multicellular organisms is aimed at the removal of unuseful cells and is essential to the development and maintenance of organism. Despite the recent progress in our understanding of plant cellular events, numerous uncertainties remain. ROS accumulation in response to various biotic and abiotic stresses has been implicated in programmed cell death. The ROS cause oxidative damage to membrane lipids, proteins and nucleic acids in cells and these intracellular changes are believed to trigger-off a variety of responses in plant cells. The ROS signal is believed to be mediated through alterations in Ca^{2+}-fluxes, redox changes, ATP depletion, membrane vulnerability, ion leakage and disruptions to cellular functioning.

Further work in this field, such as the analysis of Ca^{2+} and redox signaling, are likely to elucidate the associated molecular mechanisms responsible for regulating plant cell death and survival under various stresses. Such studies may provide new strategies to develop crop resistant to biotic and abiotic stresses (Dhariwal et al., 1998; Dhariwal and Uchimiya, 1999).

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Author Queries

Chapter No.: 17 0001088405

Queries	Details Required	Author's Response
AU1	The citation 'Kawai et al., 2000' (original) has been changed to 'Kawai and Uchimiya, 2000'. Please check if appropriate.	
AU2	The citation 'Ito and Fukuda (2002)' (original) has been changed to 'Ito and Fukuda (2000)'. Please check if appropriate.	
AU3	The citation 'Van der Biezen, 1998' (original) has been changed to 'Van der Biezen and Jones, 1998'. Please check if appropriate.	
AU4	The citation 'Hockelhoven et al., 2004' (original) has been changed to 'Hockelhoven, 2004'. Please check if appropriate.	
AU5	The spelling "Dhaliwal" has been changed to "Dhariwal" in both the citation to match the spelling in the list. Please check.	