Signaling Pathways of Aluminum-Induced Programmed Cell Death in Higher Plants

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Abstract Aluminum (Al) is the most abundant metals in the earth's crust. Al stress triggers the production of nitric oxide (NO) and hydrogen peroxide (H_2O_2). The homeostasis between NO and H_2O_2 may be a key decision point of cell survival or cell death. Al toxicity can break NO and H_2O_2 homeostasis and induce programmed cell death (PCD) in plants, which is characterized by nucleus condensation and crescent-shaped, marginalized chromatin aggregation, and DNA Ladder. This multiple programming and damaged process is mediated via two signaling pathways. One is mitochondria-dependent pathway. The excess Al toxicity-generated production of ROS leads to lipid peroxidation and induces the opening of MPTP, and then causes the release of Cyt c and finally results in PCD. Another is multi-organelle and nucleus-guided mitochondria-independent pathway, which is executed by regulating gene expressions of PCD promoter and suppressor. The promoters include senescence-associated gene (*SAG*), vacuole processing enzyme (*VPE*), poly (ADP ribose) polymerase (*PARP-1*), and *PDCD5*. Bax inhibitor-1 (*BI-1*), ACCELERATED CELL DEATH2 (*ACD2*), and LESION SIMULATING DISEASE1 (*LSD1*) all belong to the suppressor. There is a negative relationship between the occurrence of PCD and Al resistance, so the negative regulation of Al-induced PCD may be an important mechanism of Al tolerance. In this review, we highlight the newest advances about Al-induced PCD in the context of the relevant literature and enlarge our knowledge on cell death signaling pathways in plants under Al stress.

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S.K. Panda, F. Baluška (eds.), *Aluminum Stress Adaptation in Plants*, Signaling and Communication in Plants 24, DOI 10.1007/978-3-319-19968-9_4

1 Introduction

Aluminum (Al) is a major limiting factor for crop production in acid soil. When soil pH drops below 5.0, the soluble Al^{3+} is toxic to plants. The inhibition of root elongation is the initial symptom of Al toxicity. Al initially reduces cell elongation, thus decreasing root growth and causing damage to epidermal and cortical cells (Blamey et al. 2004). As a DNA-damaging agent in vivo, Al halts cell cycle progression and forces differentiation of the quiescent center. The cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of Al-dependent root growth inhibition (Nezames et al. 2012).

Extensive efforts have been made; plant species have evolved diverse mechanisms of Al tolerance, including the secretion of Al-induced organic acids, immobilization of Al at cell wall, and increasing in rhizosphere pH (He et al. 2012), but the detailed mechanisms of Al toxicity and tolerance are still poorly understood. Programmed cell death (PCD) is defined as a form of cell death involving a series of orderly processes mediated by intracellular death programs, regardless of the triggers or the hallmarks of its exhibits (Zhang and Xing 2008). PCD is a foundational cellular process in plant development and elimination of damaged cells under environmental stresses. Recently, there are some reports on Al-induced PCD in plants, such as rice (Meriga et al. 2004), barley (Pan et al. 2001; Tamas et al. 2005), tobacco (Yamaguchi et al. 1999), peanut (Zhan et al. 2009), onion (Achary et al. 2008; Andrade-Vieira et al. 2011), soybean (Rath and Barz 2000), tomato (Yakimova et al. 2007), and maize (Boscolo et al. 2003). Interestingly, Al-induced PCD process may be controlled by different signaling pathway. The manipulation of the negative regulation process of PCD may provide a novel mechanism for conferring Al tolerance (Zheng et al. 2007). To elucidate the regulatory mechanisms of Al toxicity and tolerance, herein we discuss cell death pathways during Al-induced PCD in plants by combining relevant literature.

2 Al-Induced PCD in Plants

Recent studies have described some apoptotic hallmarks that appeared upon Al treatment in plant cells (Table 1). For example, Al promoted Fe^{2+}-induced lipid peroxidation and caused death of tobacco suspension cells (Yamamoto et al. 1997). Under Al^{3+} treatment with Fe^{2+}/Fe^{3+} together, the plasma membrane integrity of tobacco suspension cells was destructed, resulting in the inhibition of cell growth (Ikegawa et al. 1998). In tobacco cultured cells, Al promoted Fe^{2+}-mediated lipid peroxidation and caused cell death, which required high concentrations of cytoplasmic Ca^{2+} and protease activities. This type of cell death-generated DNA fragmentation belonged to PCD (Yamamoto et al. 2002). When tobacco cells were treated with 50 μmol/L $AlCl_3$ for 18 h, a large number of superoxide anion and H_2O_2 arose from mitochondria. Subsequently, the membrane potential and

Table 1 The reports on Al-induced PCD in plants

Plant species	Al concentration (μmol/L)	Treatment time (h)	Characteristics	Relevant signal molecule	Reference
Nicotiana tabacum	120	18	–	H_2O_2	Yamamoto et al. (1997)
	–	8–12	–	ROS	Ikegawa et al.(1998)
	100	24	DNA degradation	ROS	Yamamoto et al. (2002)
	50	18	Cytosolic shrinkage, nucleus fragmentation	ROS	Panda et al. (2008)
	100	6	DNA degradation	ROS	Wang et al. (2009)
Hordeum vulgare	100–1000	8	DNA degradation	ROS	Pan et al. (2001)
Barley	2000	20–24	–	H_2O_2	Tamas et al. (2005)
Glycine max	15	4	Loss of cell viability	ROS	Rath and Barz (2000)
Zea mays	36	48	DNA degradation	ROS	Boscolo et al. (2003)
Oryza sativa	80	8–56	DNA degradation	–	Meriga et al. (2004)
Solanum lycopersicum	100	24	Cytosolic shrinkage, nuclear condensation	ROS	Yakimova et al. (2007)
Allium cepa	50–200	4	DNA damage	ROS	Achary et al. (2008)
Arachis hypoganea	400	96	Nucleus shrinkage, apoptotic body	–	Zhan et al. (2009, 2013, 2014)
	100	4	DNA cleavage, DAPI staining, gene expression, cytochrome C release	H_2O_2	Huang et al. (2014a, b)
Arabidopsis	500	1	–	ROS	Li and Xing (2011)

Note: ROS means reactive oxygen species

ATP content were declined. The release of cytochrome c (Cyt c) from mitochondria caused PCD (Panda et al. 2008). When tobacco was exposed to 100 μmol/L Al^{3+} for 6 h, the genomic DNA of wild-type and non-transgenic plants were degraded. Overexpression of the Ced-9 gene can inhibit Al-induced PCD in tobacco (Wang et al. 2009). After barley was treated with 0.1–1 mmol/L Al for 8 h, root tip cells generated DNA fragmentation but did not produce apoptotic bodies. Al-induced cell death of barley root tip cells may be a PCD process (Pan et al. 2001). When barley root border cells were treated with 2 mmol/L Al for 20–24 h, apoptosis-like (AL) phenomenon occurred (Tamas et al. 2005). It was showed a distinct and longtime increase in lipid peroxidation within 4 h upon transfer to an Al-containing culture medium with a calculated Al activity of 15 μM soybean cells (Rath and Barz 2000). Maize root tips were treated with 36 μmol/L Al^{3+} for 48 h; the result of TUNEL detection is positive (Boscolo et al. 2003). When rice was treated with 80 μmol/L Al stress for 8–56 h, DNA breakage occurred in root tip cells (Meriga et al. 2004). Tomato suspension cells were treated with 100 μmol/L Al for 24 h; only 67.5 % cells emitted fluorescence by FDA staining, indicating 32.5 % cells had died (Yakimova et al. 2007). When onion root cells were treated with 50–200 μmol/L Al, distinct trailing emerged from comet assay (Achary et al. 2008). Therefore, the negative regulation of Al-induced PCD may be an important mechanism of Al tolerance.

Al induced caspase-3-like activation and PCD, which provided new insight into the signaling cascades that modulate Al phytotoxicity mechanism (Li and Xing 2011). Al induced obvious PCD morphological characteristics, including nucleus condensation, crescent-shaped or oval-shaped, and similar apoptotic bodies. The difference of Al-induced PCD has a negative correlation with Al tolerance of peanut root tips (Zhan et al. 2009).

As described above, it can be seen that Al stress induces morphological changes of plant cells significantly, exhibiting distinct characteristics corresponding to PCD such as nucleus condensation and crescent-shaped, marginalized chromatin aggregation, DNA Ladder, cytochrome C release, special gene expression, etc. And apoptotic bodies are formed in some cases. The physiological aspects of Al-induced PCD also are altered, including severe damage of the mitochondrial respiratory functions, changes of the redox status and the internal structure, and tardy responses to environmental stress.

3 NO and H_2O_2 Homeostasis

Reactive oxygen species (ROS) and NO are highly reactive and diffusible molecules, and they are known to play key signaling roles in both animal and plant cells, regulating many physiological responses. NO has a strong relationship with another reactive species: hydrogen peroxide. ROS are not only toxic by products of aerobic metabolism with strictly controlled cellular levels, but they also function as signaling agents regulating many biological processes and producing pleiotropic effects.

Al treatments induced cell death possibly via a ROS-activated signal transduction pathway (Pan et al. 2001). Roots are the major sites of Al localization, and accumulation of Al promoted oxygen free radicals mediated peroxidation of membranes (Meriga et al. 2004).

ROS have become recognized as important modulators of plant PCD with emphasis on H_2O_2 (Gadjev et al. 2008). Root growth inhibition by Al is probably caused by cell death due to peroxidase-mediated H_2O_2 production (Simonovicova et al. 2004). Al-induced cell death of barley-root border cells is correlated with peroxidase- and oxalate oxidase-mediated H_2O_2 production (Tamas et al. 2005). It has established that H_2O_2 is a key player in stress and PCD responses (Gechev and Hille 2005). Low concentrations of Al stimulate the production of ROS and subsequent cell death (Yakimova et al. 2007).

Our results showed that Al stress induced ROS burst, upregulated Rboh and COX gene expression, increased mitochondrial permeability transition pore (MPTP) opening, decreased inner mitochondrial membrane potential ($\Delta\psi m$), released cytochrome c from mitochondria to cytoplasm, activated caspase 3-like protease activity. Exogenous H_2O_2 aggravated the changes caused by Al and accelerated PCD occurrence, but ROS scavenger CAT and AsA reversed the changes caused by Al and inhibited PCD production (Huang et al. 2014b). Al inhibited catalase (CAT) activity and enhanced the activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) significantly in a dose-response manner (Achary et al. 2008). Through reactive oxygen intermediates (ROI), the biphasic (hormetic) mode of action of Al that at high doses-induced DNA damage and at low nontoxic doses-conferred genomic protection was mediated (Achary and Panda 2010). Al-induced mitochondrial ROS possibly originated from complex I and III damage in the respiratory chain through the interaction between Al and iron–sulfur (Fe–S) protein (Li and Xing 2011). The specificity of the biological responses to ROS depends on the chemical identity of ROS, intensity of the signal, sites of production, plant developmental stage, previous stresses encountered, and interactions with other signaling molecules such as NO, lipid messengers, and plant hormones (Gechev et al. 2006). Hydrogen peroxide works synergistically with NO to stimulate or delay programmed cell death and assist in defense responses to pathogens (de Pinto et al. 2006; Besson-Bard et al. 2008).

NO is a freely diffusible, gaseous free radical and an important signaling molecule in animals. In plants, NO influences growth and development, and can affect plant responses to stress. Al affects mitochondrial functions, which leads to ROS production, probably the key critical event in Al inhibition of cell growth (Yamamoto et al. 2002). NO is often generated contemporaneously with H_2O_2. The effects of NO are the results of its interaction with ROS in some cases, and these interactions can be cytotoxic or protective. The presence of NO donors delays the loss of CAT and SOD that metabolize ROS, speculating that NO may be an endogenous modulator of PCD in barley aleurone cells (Beligni et al. 2002). NO plays an important role in protecting the plant against Al-induced oxidative stress (Wang and Yang 2005). The reduction of endogenous NO concentrations resulting

from inhibition of nitric oxide synthase (NOS) activity could underpin Al-induced arrest of root elongation in *H. moscheutos* (Tian et al. 2007). However, by alleviating Al-induced oxidative stress in red kidney bean roots, nitrate reductase (NR)-dependent NO production plays an important role in providing protection against Al toxicity (Wang et al. 2010). Al^{3+} induced an increase of NO in rice seedlings, proposing that NO activated multiple pathways that enhance rice adaptation to Al^{3+} toxicity (Yang et al. 2013). It is indicated that the contribution of NOS or NR-mediated NO production is dependent on plant species and environmental stimuli. NO maybe controls PCD by regulating the expression of PCD-related genes (Zhan et al. 2011). NO was first seen as punctuate foci at the cell surface, and subsequent NO was an intercellular signal that functions in cell-to-cell spread of the HR (Zhang et al. 2003).

Owing to their mobility, NO and H_2O_2 may act as signal transmission mediator of oxidative and nitrosative stress. Elevated NO levels are sufficient to induce PCD in Arabidopsis cells independent of ROS (Clarke et al. 2000). The simultaneous increase of NO and ROS activated a process of death with the typical cytological and biochemical features of hypersensitive PCD and a remarkable rise in PAL activity. Under the simultaneous generation of NO and ROS, the cellular antioxidant capabilities were also suppressed (de Pinto et al. 2002). SOD accelerates O_2^- dismutation to H_2O_2 to minimize the loss of NO by reaction with O_2^- and to trigger hypersensitive cell death through NO/H_2O_2 cooperation. The rates of production and dismutation of O_2^- generated during oxidative burst play a crucial role in the modulation and integration of NO/H_2O_2 signaling in hypersensitive reaction (HR) (Delledone et al. 2001). Al exposure caused rapid depolarization of the plasma membrane. The extent of depolarization in cells of the distal was much more extensive than in the proximal portion of the transition zone. Cells of the distal portion of the transition zone emitted large amounts of NO, and this was blocked by Al treatment (Illes et al. 2006).

There is a convergence between NO and H_2O_2 signaling, which functions at the center of cellular stress responses. In the process of normal development, plants maintain a tight NO and H_2O_2 homeostasis. When plants are subjected to environmental stress, if the balance between NO and H_2O_2 production is in favor of NO, plants show favorable stress tolerance. If the balance is in favor of H_2O_2, plants will be easily injured and even die. Al toxicity can break NO and H_2O_2 homeostasis and induce PCD in plants. Conserved negative regulators of PCD are involved in integrated regulation of cell survival and Al-induced PCD (Wang et al. 2009). Eukaryotic cells have to constantly cope with environmental cues, and cell survival or death is the only possible outcome (Cacas 2010). The homeostasis between NO and H_2O_2 is key decision point of cell survival or cell death. Meanwhile, alternative oxidase (AOX), the unique respiratory terminal oxidase in plants, not only alleviated excessive ROS accumulation but also suppressed NO concentration. So AOX plays a central role in NO and ROS homeostasis in mitochondria (Gupta et al. 2012) and was also demonstrated to play protective roles in Al-induced protoplast death (Li and Xing 2011).

4 Transcription Factors Related to Al-Induced PCD

Genetic and functional genomic studies have shown that many transcription factors (TFs) play essential roles in developmental PCD and abiotic stress PCD. Three basic helix-loop-helix transcription factors, UDT1 (bHLH164), TDR1 (bHLH5), and EAT1/DTD1 (bHLH141), are known to function in rice pollen development. bHLH142 acts downstream of UDT1 and GAMYB but upstream of TDR1 and EAT1 in pollen development. In vivo and in vitro assays demonstrated that bHLH142 and TDR1 proteins interact. Transient promoter assays demonstrated that regulation of the EAT1 promoter requires bHLH142 and TDR1. EAT1 positively regulates the expression of AP37 and AP25, which induce tapetal programmed cell death. The bHLH142 transcription factor coordinates with TDR1 to modulate the expression of EAT1 and regulate tapetal programmed cell death and pollen development (Ko et al. 2014).

It is identified that a glyoxal oxidase (GLOX1), a pectin methylesterase (VANGUARD1), and an A1 aspartic protease (UNDEAD) are direct targets of MYB80. TUNEL assays showed that when UNDEAD expression was silenced using small interfering RNA, premature tapetal and pollen programmed cell death occurred, resembling the myb80 mutant phenotype. UNDEAD possesses a mitochondrial targeting signal and may hydrolyze an apoptosis-inducing protein(s) in mitochondria (Phan et al. 2011).

WRKY transcription factors have been implicated in various transcriptional programs, including biotic and abiotic stress responses, growth, and development (Pandey and Somssich 2009; Rushton et al. 2010, 2012; Van Aken et al. 2013). As the most widely discussed H_2O_2-inducible representative of the family, WRKY52 is a senescence-related factor and its overexpression leads to accelerated senescence (Miao et al. 2004). ORESARA1 SISTER1 (ORS1), a member of the NAC transcription factor (TF) family, triggers expression of senescence-associated genes through a regulatory network that may involve cross-talk with H_2O_2-dependent signaling pathways (Balazadeh et al. 2011). A C2H2-type zinc finger transcription factor ART1 (for Al resistance transcription factor 1), which specifically regulates the expression of genes related to Al tolerance in rice (*Oryza sativa*), was identified. ART1 regulates 31 genes implicated in Al tolerance in both internal and external detoxification of Al at different cellular levels, including STAR1 and 2 in rice (Yamaji et al. 2009). It had been successful in identification of cis-acting element of ART1, which is present in the promoter regions of 29 genes out of 31 genes regulated by ART1 (Tsutsui et al. 2011). It is regret that there are no reports on the transcription factors related to Al-induced PCD.

5 The Genes Related to Al-Induced PCD

The genes controlling the genotypic variation in Al^{3+} tolerance have been cloned such as ALMTs (Aluminum-activated malate transporter) and MATEs (multidrug and toxic compound extrusion), which have been successfully expressed in plants (wheat, barley, Arabidopsis, and rice) as well as tobacco suspension cells (Ryan et al. 2011). STOP1 (sensitive to proton rhizotoxicity1) and ART1 (Al^{3+} resistance transcription factor 1) share significant sequence similarity and appear to act as transcription factors to enhance the expression of a range of genes in Al^{3+}-treated roots. STOP1 is a Cys2His2-type zinc-finger protein belonging to a family of transcription factors and localizes to the nucleus. The stop1 mutant is also sensitive to Al^{3+} (but not other metal ions). STOP1 likely functions as a transcription factor that regulates the expression of proton and Al^{3+} responsive genes. ART1, similar to STOP1, belongs to the family of Cys2His2-type zinc-finger transcription factors. ART1 regulates the expression of multiple Al^{3+}-tolerance genes in rice such as *OsFRDL4*, *STAR1/2*, *Nrat1* (Nramp aluminum transporter 1), and *OsALS1* (Delhaize et al. 2012).

Natural senescence is a genetically determined cell death progress, characterized by upregulation of many senescence-associated genes (SAGs) (Rosenvasser et al. 2006). A hypothesis was proposed that SAGs can serve as integrators of different signaling pathways that control environmental responses (Balazadeh et al. 2010). We isolated *AhSAG* (a senescence-associated gene) from cDNA library of Al-stressed peanut with PCD, which Open reading frame (ORF) of AhSAG is 474 bp, encoding a SAG protein composed of 157 amino acids. The *AhSAG* was transferred into tobacco. Compared to the control and the antisense transgenic tobacco plants, the fast development and blossom of the sense transgenic plants happened to promote senescence. The ability of Al tolerance in sense transgenic tobacco was lower than in antisense transgenic tobacco according to root elongation and Al content analysis. The expression of AhSAG-GFP was higher in sense transgenic tobacco than in antisense transgenic tobacco. It showed that *AhSAG* can induce or promote the occurrence of PCD in plants (Zhan et al. 2013).

In animal, one group of cysteine proteinases, the cysteine-dependent aspartate-specific proteinases (caspases), are involved in a proteolytic signaling cascade that controls apoptosis. The similar apoptotic caspase cascade has not been uncovered in plants, but other proteolytic enzymes involved in PCD had been found, which are localized in different compartments of plant cells: the cytoplasm (metacaspases), the vacuoles (VPE), and the intercellular fluid (phytaspases). Vacuolar processing enzyme (VPE) is a cysteine-dependent protease responsible for caspase-1 activity in plant and is localized in plant cell vacuoles (see Fig. 1), where it participates in the processing of vacuolar proteins, and its physiological role has been most extensively investigated (Hatsugai et al. 2006). The Arabidopsis genome has four *VPE* homologues traditionally distributed into seeds, *βVPE* and *δVPE*, and vegetative tissues, *αVPE* and *γVPE*. *γVPE* is a vacuolarlocalised cysteine protease with a caspase-1 like activity involved in the activation and maturation of downstream

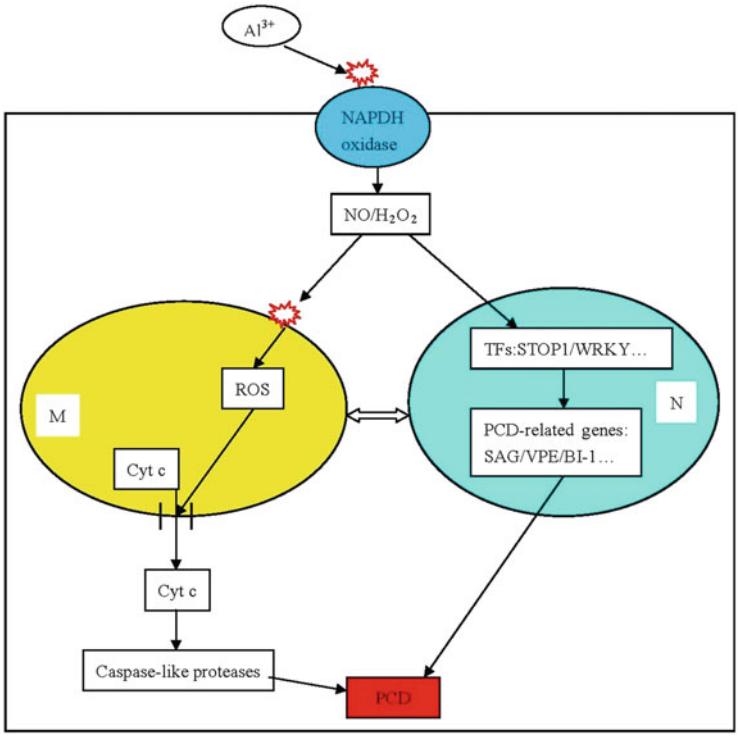

Fig. 1 A schematic illustration of possible signaling pathways of aluminum-induced programmed cell death in higher plants

vacuolar hydrolytic enzymes that trigger hypersensitive cell death and tissue senescence. This work provides evidence that *γVPE* is strongly expressed in *Arabidopsis* guard cells and is involved in water stress response (Albertini et al. 2014). VPE functions as executioner of plant PCD through disrupting the vacuole in pathogenesis, seed development, and abiotic stress such as heat shock (Li et al. 2012). Real-time quantitative RT-PCR indicated that four VPE genes (*NtVPE-1a*, *NtVPE-1b*, *NtVPE-2*, *NtVPE-3*) were more or less enhanced by the Al exposure over the control levels. Especially, the expressions of the NtVPE-1a and the NtVPE-1b were significantly enhanced, by 2.5-fold under the Al stress. It is concluded that an enhancement of VPE activity by Al is controlled at transcriptional level and is a key factor leading to a loss of integrity of the plasma membrane and a loss of growth capacity (Kariya et al. 2013).

Caenorhabditis elegans apoptotic suppressor Ced-9, a Bcl-2 homologue, inhibited both the Al-induced PCD and Al-induced activity of caspase-like vacuolar processing enzyme (VPE) in tobacco. Furthermore, Ced-9 significantly alleviated Al inhibition of root elongation, decreased Al accumulation in the root tip, and greatly inhibited Al-induced gene expression in early response to Al, leading to enhancing the tolerance of tobacco plants to Al toxicity. It suggests that Ced-9

promotes Al tolerance in plants via inhibition of Al-induced PCD, indicating that conserved negative regulators of PCD are involved in integrated regulation of cell survival and Al-induced PCD (Wang et al. 2009).

PpBI-1 can attenuate Al-induced PCD and enhance Al tolerance in transgenic yeast (Zheng et al. 2007). The programmed cell death 5 (*PDCD5*) gene encodes a protein that shares significant homology with the corresponding proteins of species ranging from yeast to mice (Liu et al. 1999). Overexpression of OsPDCD5 genes induces PCD in rice (Attia et al. 2005). As a molecular chaperone, mitochondrial HSP70 may be involved in PCD initiation by reducing $\Delta\psi m$ in mitochondrial outer membrane (Chen et al. 2009).

6 The Signaling Pathways of Al-Induced PCD

Over the years, forward and reverse genetic screens have uncovered numerous regulators of PCD in plants. However, to date, molecular networks are far from being deciphered (Cacas 2010). Inside the cell, the compartments that produce the highest amounts of ROS and NO are chloroplasts and mitochondria. The mitochondrial electron transport chain harbors electrons with sufficient free energy to directly reduce O_2 which is considered the unavoidable primary source of mitochondrial ROS generation. It was suggested that the mitochondrial transmembrane potential loss and the changes in distribution and mobility of mitochondria, as well as the production of ROS, play important roles during UV-induced plant PCD (Gao et al. 2008).

Based on understanding of related knowledge and NO signaling network proposed by us (He et al. 2014), a new mechanism of Al-induced PCD is proposed in Fig. 1. Al-induced PCD may be mediated via two divergent signaling pathways. One is mitochondria-dependent pathway. Al stress provokes the activity of NADPH oxidase, triggers ROS burst, ROS burst works as a signal of PCD production, opens MPTP, releases cytochrome c, activates caspase 3-like protease, and then promotes PCD occurrence (Huang et al. 2014b). Through NO/H_2O_2 cooperation, SOD accelerates O_2^- dismutation to H_2O_2 to minimize the loss of NO by reaction with O_2^- to trigger hypersensitive cell death (Delledone et al. 2001).

Another is multi-organelle-participated and nucleus-guided mitochondria-independent pathway, which is executed by regulating gene expressions of PCD promoter and suppressor, then vacuolar collapse, a loss of plasma membrane integrity, and eventually reaching to a loss of growth capacity. The promoter includes senescence-associated gene (*SAG*), vacuole processing enzyme (*VPE*), poly (ADP ribose) polymerase (*PARP-1*), and programmed cell death 5 (*PDCD5*). Bax inhibitor-1 (*BI-1*), ACCELERATED CELL DEATH2 (*ACD2*), and LESION SIMULATING DISEASE1 (*LSD1*) all belong to the suppressor. Al is able to not only generate a signal cascade but also modulates other signal cascades generated by other types of stress in plants (Poot-Poot and Hernandez-Sotomayor

2011). The final output of the cascade depends on the intensity of Al stress, NO/H_2O_2 signaling, and two-way communication between two signaling pathways.

As a stress sensor of death signals and a dispatcher of PCD, mitochondria can serve in plant and animal cell death (Jones 2000). In contrast, the part played by mitochondria in the death of plant cells has little attention. High Al^{3+} concentration treatment induced mitochondrial permeability transition pore (MPTP) opening, increased mitochondrial membrane permeability, Cyt c released into the cytoplasm, activated caspase 3-like protease, which might induce PCD in root tip (Zhan et al. 2009; Huang et al. 2014b). Al treatment and oxidative stress in the sensitive maize line induced cell death in root tips cells (Boscolo et al. 2003). Al enhances ferrous ion (Fe^{2+})-mediated lipid peroxidation which is the primary factor leading to cell death in nutrient medium in tobacco cells (Yamaguchi et al. 1999). Spent pot liner (SPL) is solid waste from the Al industry. This toxic agent, consisting of cyanides, fluorides, organics, and metals, leads to cell damage and disturbance (Andrade et al. 2010). SPL induces apoptosis-like PCD in root meristem cells of *Allium cepa* (Andrade-Vieira et al. 2011). NO can ameliorate remarkably mitochondrial respiratory dysfunction resulted from Al stress (He et al. 2006). Al induced oxidative burst at the cell surface through up- or downregulation of some of the key enzymes of oxidative metabolism ultimately resulting in oxidative stress leading to DNA damage and cell death in root cells of *Allium cepa* (Achary et al. 2008).

Mitochondria are the main target for oxidative damage to proteins under well-irrigated and drought conditions (Bartoli et al. 2004). As a semiautonomous organelle, mitochondrion is a common factor that integrates NO/H_2O_2 signaling. Mitochondria constitute a major source of ROS and have been proposed to integrate the cellular responses to stress. Oxidative stress increased mitochondrial electron transport, resulting in amplification of H_2O_2 production and cell death. The increased generation of H_2O_2 also caused the opening of the mitochondrial transmembrane potential (MTP) and the release of Cyt c from mitochondria (Tiwari et al. 2002; Huang et al. 2014b). Exposure to H_2O_2 caused the opening of permeability transition pores in the inner mitochondrial membrane. Cytosolic Cyt c plays an essential role in the execution of apoptosis (Takeyama et al. 2002). We found that $\Delta\Psi m$ loss is a common early marker in plant PCD; mitochondrial Cyt c release is an obligatory step in PCD control also (Huang et al. 2014b). Mitochondrial swelling and MTP loss, as well as the generation of mitochondrial ROS, play important roles in Al-induced PCD (Li and Xing 2011). Al toxicity affects severely the mitochondrial respiratory functions and alters the redox status studied in vitro and also the internal structure, which seems to cause finally cell death in tobacco cells (Panda et al. 2008).

However, it has recently been shown that PCD can still occur even when the mitochondria are removed, revealing that there is a mitochondria-independent signaling pathway in nucleus. Proteolytic cleavage of nuclear lamin was conserved in plant PCD (Sun et al. 1999). The nuclear matrix largely remained intact during the course of apoptosis, maintaining the integrity of apoptotic cells and connecting the apoptotic bodies and apoptotic nucleus (Zhao et al. 2001). As one of the

hallmarks of apoptosis, chromatin condensation is regulated by nucleoplasmin (Lu et al. 2005). NO and H_2O_2 cause an induction of caspase-like proteases previously characterized in physiological nucellar PCD (Lombardi et al. 2010). Using physiological, biochemical, and genetic approaches, we recently demonstrated that *AhSAG* could induce or promote Al-induced PCD (Zhan et al. 2013). Although VPE is structurally unrelated to caspases, plants have evolved a regulated cellular suicide strategy that is mediated by VPE and the cellular vacuole (Hatsugai et al. 2004). Al induced the activity of caspase-like VPE, a crucial executioner of PCD in tobacco (Wang et al. 2009). *Bcl-2* overexpression suppresses H_2O_2-induced PCD via *OsVPE2* and *OsVPE3*, but not via *OsVPE1* and *OsVPE4*, in rice (Deng et al. 2011).

The Arabidopsis *PARP-1* shows high homology to human *PARP-1*, and its activity is inhibited by the caspase-3 inhibitor (Ac-DEVD-CHO). By regulating synthesis of PAR, *PARP-1* processes diverse signals and directs cells to specific fates (DNA repair, energy depletion, or cell death) (Luo and Kraus 2012). Because the PDCD5 protein can translocate rapidly to the nucleus in cells undergoing apoptosis, overexpression of the *OsPDCD5* gene induces PCD in rice (Attia et al. 2005). *PpBI-1* (*Phyllostachys praecox*) inhibits Al-induced PCD and promotes Al tolerance in yeast (Zheng et al. 2007). The C-terminal hydrophilic region of BI-1 is essential for the inhibition of cell death. H_2O_2-mediated cell death was suppressed in tobacco BY-2 cells overexpressing *AtBI-1* (Kawai-Yamada et al. 2004). The *Arabidopsis* ACD2 protein protects cells from PCD caused by endogenous porphyrin-related molecules like red chlorophyll catabolite or exogenous protoporphyrin IX (Pattanayak et al. 2012).

LSD1 is an important negative regulator of PCD in Arabidopsis. The loss-of-function mutations in LSD1 cause run away cell death triggered by ROS (Li et al. 2013). Although caspases are proteases that act as key components of animal apoptosis, plants have no orthologous caspase sequences in their genomes. Metacaspase-8 is part of an evolutionary conserved PCD pathway activated by oxidative stress, so metacaspases may be the functional homologues of animal caspases in these organisms (He et al. 2008). The prolonged activation of the mitogen-activated protein kinase (MAPK) pathway in cells could disrupt the redox balance, which leads to the generation of ROS and eventually cell death (Ren et al. 2002). The PCD-related genes are mediated by TFs, redox changes, MAPK cascades, microRNAs, and their interactions with each other.

Moreover, Cyt c induced in vitro apoptosis of carrot nucleus, indicating there is a signal communication between mitochondria and nucleus (Zhao et al. 1999). Chloroplasts may be involved in mediating certain types of plant PCD (Chen and Dickman 2004). Doyle et al. (2010) found that chloroplasts can play a significant role in Al-PCD regulation. Distinct organelles sense a broad range of stimuli, if necessary, engage cell death signaling pathways. The endomembrane system (ES) seems to harbor a significant number of cell death mediators (Cacas 2010). *AtLrgB*, which encodes a homolog of the bacterial membrane protein LrgB, functions against cell death (Yamaguchi et al. 2012).

7 Conclusions and Perspectives

In conclusion, Al stress not only triggers the production of NO and H_2O_2 but also induces PCD by breaking their homeostasis. Al-induced PCD is characterized by nucleus condensation and crescent-shaped, marginalized chromatin aggregation, and DNA Ladder. In the light of relevant literature, Al toxicity initiates PCD via two signaling pathways. One is mitochondria-dependent pathway. The excess Al toxicity-generated production of ROS lead lipid peroxidation, induce the opening of MPTP, cause the release of Cyt c, activate caspase 3-like protease, and finally result in PCD. Another is mitochondria-independent pathway existing in nucleus. It is a multiple organelle-participated and nucleus-guided process, which is executed by regulating expressions of PCD-related genes, such as *SAG*, *VPE*, *BI-1*, *ACD2*, *PDCD5*, and *LSD1*. Since there is a negative relationship between the occurrence of PCD and Al-resistance in peanut (Zhan et al. 2013), the negative regulation of Al-induced PCD may be an important mechanism of Al tolerance.

Although researches on signaling molecules, related proteins, and genes of Al-induced PCD in plants have made some progress, its precise mechanism is still unclear. For example, how is the relationship between PCD occurrence and Al tolerance in different plants? Whether the mitochondria lie in the control center of Al-induced PCD? What kinds of species are signaling factors related to Al-induced PCD? Whether common regulatory pathway or mechanism exists? What are the similarities and differences of Al-induced PCD mechanism at the molecular level? Which kinds of transcription factors are related to Al-induced PCD? The role of nuclease and specific protease in Al-induced PCD is still unknown. Deep research on molecular mechanism and regulatory pathways of Al-induced PCD help to elucidate the mechanisms of Al toxicity and Al tolerance in plants, providing opportunities for enhancing the Al^{3+} resistance of plants by marker-assisted breeding and through biotechnology.

Acknowledgements We apologize to many of our colleagues for not being able to cite many exceptional articles due to space limitations. This work is supported by grants from the National Natural Science Foundation of China (No. 31260296 and 30960181) and 2011 Guangxi Innovation Program for Graduates (GXU11T31076).

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