# Chapter 5
# Manipulating Programmed Cell Death Pathways for Enhancing Salinity Tolerance in Crops


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**Abstract** One of the key challenges for researchers is to obtain a deeper understanding of the strategies and mechanisms of plant adaptation to environmental stress that help overcome the limitations associated with climate change and loss of biodiversity. In this context, tolerance to salinity stress is one of the main abiotic factors constraining the plant growth, and production is of special importance. Programmed cell death (PCD) plays a protective role against biotic and abiotic stresses. PCD might play an important role in the maintenance of normal tissue homeostasis, regulation of cell metabolism, and remodeling of tissues after injury and infection as well as the elimination of damaged cells. Salinity stress induces an alteration in chloroplasts, mitochondria, cytoplasm, plasma membrane (PM), endoplasmic reticulum (ER), Golgi apparatus, vesicle formation and trafficking, and vacuoles formation which may result in PCD in plants. The overexpression of pro-survival genes including anti-apoptotic genes and those involved in suppression of apoptosis genes in the transgenic plants to enhance abiotic stress tolerance has been the subject of a number of investigations, particularly in the context of salinity tolerance. Therefore, the development of transformed plants for resistance to apoptosis could be an effective approach to improving salinity tolerance, while the use of complementary techniques like RNA-interfering (RNAi)-mediated gene knockdowns has been shown to be an interesting and appealing alternative. The objective of this review is to summarize the current state of knowledge on improving salinity tolerance in crop plants through manipulation of PCD pathways.

**Keywords** Abiotic stress · Molecular networks · Salt · Transformation · Vacuolar processing enzyme

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V. Kumar et al. (eds.), *Salinity Responses and Tolerance in Plants, Volume 2*,
https://doi.org/10.1007/978-3-319-90318-7_5

## Abbreviations

| | |
|---|---|
| AIF | Apoptosis-inducing factor |
| AL-PCD | Apoptotic-like PCD |
| ASPP | Apoptosis-stimulating proteins of p53 |
| ACD | Autophagic cell death |
| BAG | Bcl-2-associated athanogene |
| Bak | BCL-2 antagonist/killer-1 |
| Bax | Bcl-2-associated X protein |
| Bcl-2 | B-cell lymphoma2 |
| Bcl-xl | BCL-2-like 1 |
| $Ca^{2+}$ | Calcium ion |
| ER | Endoplasmic reticulum |
| FB1 | Fumonisin B1 |
| GORK | Guard cell outward-rectifying $K^+$ channel |
| $H_2O_2$ | Hydrogen peroxide |
| HR | Hypersensitivity |
| IAP | Inhibitor of apoptosis |
| $K^+$ | Potassium ion |
| MAPK | Mitogen-activated protein kinase |
| Mcl-1 | Myeloid cell leukemia-1 |
| $Na^+$ | Sodium ion |
| NADPH | Nicotinamide adenine dinucleotide phosphate hydrogen |
| PM | Plasma membrane |
| RNAi | RNA interfering |
| ROS | Reactive oxygen species |
| PCD | Programmed cell death |
| PLC | Phospholipase C |
| SKOR | Outward-rectifying $K^+$ channel |
| VPE | Vacuolar processing enzyme |

## 5.1 Introduction

Abiotic stress threatens staple crop production, coupled with the expanding world population necessitate not only efficient breeding strategies for developing abiotic stress tolerance crop plants but also the extension of plant production into the marginal regions including saline soil and water (Arzani and Ashraf 2016). Soil or water salinity is one of the key abiotic stresses that cause plant growth and yield reduction worldwide (Arzani 2008). Abiotic stress can be sensed and appropriate responses triggered implicating changes in growth, development, and metabolism (Conde et al. 2011). Plants' response to salinity stress represents the sum of numerous parallel-distributed processes that act to alleviate hyperosmolarity and reestablish ionic homeostatic conditions in cells (Arzani and Ashraf 2016). Programmed cell

death (PCD) is also among the evolved plant strategies to overcome these adverse conditions. PCD operates during growth and development as well as in response to various hostile environmental conditions. In this way the removal of damaged and superfluous cells can be facilitated; thus, cellular differentiation and homeostasis are supported in plants (De Pinto et al. 2012).

Therefore, PCD plays not only a protective role against abiotic and biotic stresses but also a major role in plant development. PCD is a highly coordinated process with series of steps involving specific nucleases and proteases and results in the selective elimination of the cells. In animals, autophagy, apoptosis, and programmed necrosis are the three major PCD forms, clearly characterized by their morphological features (Bialik et al. 2010; Ouyang et al. 2012). Autophagy is usually defined by the accumulation of autophagic vacuoles. Chromatin condensation, the formation of apoptotic bodies and nuclear fragmentation by the caspases as the executioners of apoptosis are the hallmarks of apoptosis. A more passive form of PCD is necrosis, which is distinguished by the presence of PM rupture and cytoplasmic swelling.

Autophagic cell death (ACD) is one of the characterized types of PCD. Autophagy process is initiated with the generation of double membrane-bound autophagosomes, encompassing cytoplasmic organelles and macromolecules, headed for recycling (Huett et al. 2010). There is increasing recognition that autophagic cells commit suicide to prevent excessive stress by undertaking cell death, which discriminates from programmed necrosis and apoptosis (Bialik et al. 2010). Nonetheless, autophagy regulates an enormous number of physiological and pathological functions such as cell differentiation, infections, starvation, cell survival, and death (Liu et al. 2010; Michaeli et al. 2016). The role of autophagy in cell death has been reviewed by Minina et al. (2014). In addition, recent advances in plant autophagy regarding mechanisms of selective autophagy, regulation of autophagy, and role of autophagy in recycling and availability of nutrients have recently been reviewed by Michaeli et al. (2016).

"Apoptosis," as the second form of PCD, comes from a Greek root word that has been used to refer to "dropping off" the leaves or petals from a tree (Kerr et al. 1972). Given the definition roots, apoptosis is likely the most frequent type of PCD, while the biological impact of other non-apoptotic types may also be a driving force of the PCD especially in plants. Apoptosis is characterized by morphological alterations of nucleus and cytoplasm including cell shrinkage, pyknosis (DNA condensation), and karyorrhexis (nuclear fragmentation) as well as biochemical changes such as internucleosomal cleavage of DNA, a number of intracellular substrate cleavages by specific proteolysis, and phosphatidylserine externalization (Ouyang et al. 2012).

Programmed necrosis as the third type of PCD contributes to cell swelling, cell lysis, and organelle dysfunction (Wu et al. 2012). Therefore, PCD may have a role in the maintenance of tissue homeostasis, regulation of cell metabolism, and remodeling of tissues after injury and elimination of damaged cells (Wynn et al. 2013). In contrast to the wealth of knowledge regarding the molecular mechanisms of PCD, in plants the molecular networks regulating PCD are still in their infancy, and

information on this topic is scarce. This is in spite of the abundance and the importance of PCD throughout plant life span occurring as a conspicuous part of development (dPCD) as well as a response to abiotic and biotic stresses (ePCD) (Lam 2004; Huysmans et al. 2017). Although plants react differently to various abiotic stresses, the initial recognizing and induction of reactive oxygen species (ROS) generation are a common set of response to abiotic factors in all plant species (Sewelam et al. 2016). Nonetheless, production of ROS is a crucial factor in plant stress response and is also associated to in signaling of PCD (Chen et al. 2009a; Kumar et al. 2016).

In animals, ICE-/CED-like family proteases, named caspases, play a central role in PCD such as apoptosis and pyroptosis (Green 2011). In spite of the absence of caspases (abbreviation of cysteinyl aspartate-specific proteases) in plants, the metacaspases were postulated as the functional caspase homologs in plants (Bonneau et al. 2008). In recent years, significant knowledge has been gained in these areas including the characterization of two PCD types: vacuolar PCD and necrotic PCD. The apoptotic cells can be eliminated in the animal using macrophages, whereas in plant lytic vacuoles progressively engulf and digest the cytoplasmic content during vacuolar cell death. On the other hand, necrosis is an alternative form of cell death which is triggered by severe stress and characterized by mitochondrial dysfunction, premature rupture of the plasma membrane, and organized cell disassembly. Vacuolar processing enzyme (VPE) is a plant cysteine proteinase that is mediator driving the execution of various PCD and is considered as a counterpart of animal caspase 1 (Hatsugai et al. 2015).

Climate change and biodiversity loss create new challenges for developing dynamic strategies of plant adaptation to the changing environment. Stress-induced PCD markedly influences plant growth and yield, and it is an important threat to agriculture production (Mittler and Blumwald 2010). The applied and basic research on stress-induced PCD and stress responses, with the eventual goal of manipulating them for practical use, are incredibly challenging areas that attract the growing interest. Therefore, research on PCD-induced abiotic stress and stress responses in plants has strengthened significantly during the past years, and thereby understanding of regulatory mechanisms and knowledge of the immunity role will undoubtedly help to reach the eventual goal to lessen yield losses (Petrov et al. 2015; Wang et al. 2015). The objective of this review is to summarize the current state of knowledge on improving salinity tolerance in crop plants through manipulation of PCD pathways.

## 5.2 PCD in Response to Abiotic Stress

Plants tolerate the adverse environmental conditions by employing various adaptation mechanisms including toxin exclusion and dramatic amelioration of susceptibility (hypersensitivity) where the abiotic stress is extreme. The monitored level of applied heat stress-induced PCD in plant cells, where heat shock could be responsible for the cell death morphology, is reported in Arabidopsis (Hogg et al. 2011),

tobacco (Vacca et al. 2004), soybean (Zuppini et al. 2006), maize (Wang et al. 2015), and lace plant (Dauphinee et al. 2014). PCD in plant species has been induced by low or high temperature in tobacco (Koukalova et al. 1997), cucumber (Balk et al. 1999), *Arabidopsis thaliana* (Swidzinski et al. 2002), and maize (Wang et al. 2015).

Plant symptoms illustrating either undesirable or desirable response to salinity stress can be visually rated in the field. Nevertheless, a reduction in growth which is manifested by leaf burn and necrotic lesions on the leaves is a well-known indicator of exposure of plants to salinity (Tanou et al. 2009). It is suggested that leaf necrosis could be caused by the failure of the cells to avert the accumulation of $Na^+$ ions into the cytoplasm (Greenway and Munns 1980); in other words, leaf necrosis may be a symptom of the breakdown of ionic regulation (Subbarao and Johansen 1994). In barley, Patterson et al. (2009) compared two barley cultivars (Sahara and Clipper) exposed to 100 mM NaCl treatment and observed that "Sahara" cultivar had significantly less leaf necrosis and higher leaf $Na^+$ concentrations than "Clipper," concluding that "Sahara" has a higher tolerance to accumulated $Na^+$. However, despite a general consensus attributing leaf necrosis to an undesirable reaction in plant salinity stress, it is probably most disputable, and it could also be considered as the fundamental lack of knowledge about the reaction at the cellular level and entirely limited to macroscopic observations. Nevertheless, pathogen-induced HR cell death is one of the most efficient plant defense strategies, whereas pathogen- secreted toxin-induced cell death is a necrotrophic pathogen tactic for infection. Interestingly, although distinct mechanisms may regulate toxin-induced cell death and pathogen-induced cell death, both were mediated by the same VPE (Kuroyanagi et al. 2005).

Leaf margin, leaf tip burn, and leaf necrosis are among the plant responses to drought stress which can be found at the late vegetative stage. It was suggested that drought-induced leaf necrosis can be illustrated by the lack of anthocyanin pigmentation (Rosenow et al. 1983). Therefore, leaf necrosis is considered distinctly different from that of the disease symptom, where leaf necrosis is known as desirable plant reaction of host resistance named as "hypersensitivity (HR)." HR is a plant-specific PCD which is essential for defense response to restrict the spread of pathogens. Apoptosis is generally regarded as a critical physiological cell death program required for the tissue homeostasis as well as an active suicidal response to various pathological or physiological stimuli in the mammalian organism (Kabbage et al. 2017). Among the several cell death pathways that have been postulated, apoptotic-like PCD (AL-PCD) seems to be an interesting operational mode in plants leading to a corpse morphology that is similar to the apoptotic morphology perceived in animal cells (Reape and McCabe 2008). It is now established that AL-PCD is an essential cellular process in plants that have a crucial role in the developmental, stress-induced, and senescence processes as well as in response to pathogen infection (Lam et al. 1999). Apart from the developmental and biotic stimuli, it has been shown that AL-PCD is induced by abiotic stresses such as high-fluence UV radiation and heat stress (Foyer and Noctor 2005; Doyle et al. 2010).

Caspases are either involved or not involved in PCD. Accordingly, PCDs can be categorized into two groups, caspase-independent and caspase-dependent PCD

(Kroemer and Martin 2005). Apoptosis is entirely contingent upon caspase activation and thus caspase-dependent PCD represents typical apoptosis. Caspase-independent mechanism of cell death comprises paraptosis, autophagy, necrosis-like PCD, apoptosis-like PCD, and mitotic cataclysm. The non-caspase PCD was found to be associated with caspase-independent elimination, including the use of mitochondrial protein apoptosis-inducing factor (AIF) (Cande et al. 2002; Kroemer and Martin 2005; Zanna et al. 2005). Analysis of the *Arabidopsis* genome indicated the incidence of five close homologs of AIF which detected monodehydroascorbate reductases (MDARs) (Lisenbee et al. 2005) while AIF initially characterized in mammalian mitochondrial DNA (Susin et al. 1999). Of AL-PCD regulation especially relevant to plant cells is the affirming dual target sites of MDAR that is to both chloroplasts and mitochondria.

In plants, the role of mitochondrial proteins triggering cell death is still in its infancy and debatable (Reape and McCabe 2010). However, a pivotal role of the mitochondrion in plant PCD has also been implicated in plant responses to salinity stress (Yao et al. 2004; Lin et al. 2006; Chen et al. 2009b; Wang et al. 2010; Monetti et al. 2014; Hamed-laouti et al. 2016). ROS produced from the electron transport chain in mitochondrion causes dysfunction of mitochondrial lipids and proteins (Yao et al. 2004) leading to the opening of a nonspecific pore in the inner mitochondrial membrane, also called the permeability transition pore (PTP) and release of "caspase-like" proteins (Yao et al. 2004; Reape and McCabe 2010; Sirisha et al. 2014). The dysfunction of mitochondria has been proposed as a prerequisite for the establishment of NaCl-induced PCD in several plant species comprising both glycophyte (*A. thaliana*, rice, tobacco) and halophyte (*Cakile maritima*, *Thellungiella halophila*) (Lin et al. 2006; Chen et al. 2009b; Wang et al. 2010; Monetti et al. 2014; Hamed-laouti et al. 2016). There are considerable evidence and speculation that interaction between ROS and antioxidants would supply a boundary for the environmental metabolic signals mediating activation of the acclimation of the cells to stress or alternatively induction of PCD (Foyer and Noctor 2005).

A dual biological role for ROS might be attributed to the leaf senescence including regulation of the expression of senescence-associated genes and elevation of the program of cell death by direct oxidizing target macromolecules. Interestingly, taking in account the chloroplasts is one of the sources of ROS production in plants (Doyle et al. 2010) would help to resolve the question as to what extent the PCD reaction is responsive to the environmental stimuli in the plant kingdom. In addition, photoreduction of oxygen and energy transfer from triplet excited chlorophyll to oxygen, respectively, are responsible for generating superoxide radicals ($O^{-2}$) and singlet oxygen ($^1O^2$) in chloroplasts (Kim et al. 2012). The PCD is induced with increasing singlet oxygen ($^1O^2$) concentration in chloroplasts, but the output of $1O^2$-mediated chloroplast leakage and liberate of chloroplastic proteins to the cytosol on the $1O^2$-mediated collapse of cells needs to be elucidated.

PCD, a genetically controlled cell response, has evolved under selective pressure and thus should be advantageous to the plant. Despite the recent progress in the understanding expression of the ROS-responsive genes which induced in response to abiotic and biotic stress, many challenges remain, particularly with regard to the

beneficial effects of the ROS-dependent genes influencing PCD on plant growth and resistance to both abiotic and biotic stresses. Hence, it appears possible that induction of the ROS-dependent PCD pathway in plants can be part of physiological changes that normally occur during an acclimation response to enhance stress resistance.

PCD has been perceived traditionally as a vital protective mechanism for disease resistance in plants. Today, it appears that PCD plays a fundamental role in the regulation of much more diverse cellular functions, such as in response to biotic and abiotic stress as well as developmental processes (see a recent review by Huysmans et al. 2017). It should be acknowledged that since the dissection of the PCD at the whole-plant level is difficult, most of the attempts have been made at in vitro cell assays. Here a new scenario for the biological roles of PCD at the whole-plant level to facilitate the possible explanation contributing to the induction of PCD in response to abiotic stress is presented. The results of assessment of abiotic stress tolerance in the $C_4$ model plant, *Setaria viridis* (L.) Beauv. accessions originated from diverse geographical areas of the world, a portion of which has been published elsewhere (Saha et al. 2016), suggested to us that PCD might have occurred in response to salinity stress (Saha et al. unpublished data). Interestingly, only one accession showed leaf necrosis after 4 weeks of treatment at 300 mM NaCl concentration (Fig. 5.1) and astonishingly ranked as one of the most salinity-tolerant genotypes. Further in vitro positron emission tomography (PET) study showed a clear difference in $Na^{22}$ uptake and transport in this accession compared to a sensitive accession (Ariño-Estrada et al. 2017). However, observations at the cellular level are more pertinent for assessing the possible role of PCD in salinity tolerance than

**Fig. 5.1** Leaf necrosis resulted from 300 mM NaCl treatment for 4 weeks in one of the *Setaria viridis* (L.) Beauv. accessions

those at the whole-plant level. It is also important to note that leaf necrosis was observed at the reproductive growth stage, while the PET imaging has been conducted at the seedling stage. The inconsistency with the previously characterized leaf necrosis, as being regarded typical for the sensitive plant to salinity stress, can be explained by developmental stage differences in high levels of NaCl accumulation occurred in the leaf cells at either the reproduction (present study) or the vegetative/seedling stage (previous studies). In addition, a complex combination of differential expression of genes encoding the photosynthetic enzymes and anatomical characterization was functionally essential for evolving more effective photosynthetic mechanism in the $C_4$ plants. $C_4$ plants exhibit higher adaptation to tropical regions than $C_3$ plants and assumed to have an evolutionary adaptation in hot areas of the world in response to diminishing ambient $CO_2$ concentration (Sage 2004). Therefore, this finding inspires us to look for an alternative interpretation. Further work on the macroscopic, microscopic, and molecular aspects of the salt-tolerant leaf necrosis structure is underway to test the hypothesis that leaf necrosis might be a favorable plant response (i.e., HR) to salinity.

As illustrated in Fig. 5.2, plant cells undergoing PCD exhibit the following features: condensation of the cytoplasm and the nucleus, the retraction of the plasma membrane from the cell wall, loss of membrane integrity, DNA laddering, release of cytochrome c from mitochondria, increase in activity of the proteases of caspase-1-like and caspase-3-like, and alterations in the $K^+$ efflux and ion homeostasis (Wang et al. 2010; Poor et al. 2013; Reape and McCabe 2013; Reape et al. 2015).

## 5.3 PCD in Response to Salinity Stress

Although to date no report on HR-like response has been documented for plant salinity tolerance, several researchers have investigated PCD at the cellular level. The influences of NaCl stress primarily on chloroplasts, mitochondria, cytoplasm, plasma membrane (PM), endoplasmic reticulum (ER), Golgi apparatus, vesicle formation and trafficking, and vacuoles have been investigated in plants. The degradation of the inner chloroplast membrane due to the NaCl-induced stress on the ultrastructure of plant leaves has been reported by Hernandez et al. (1995). The disintegration of organellar membranes (particularly the degradation of thylakoid membrane of chloroplast) in cells was found to be one of the major effects of salinity stress (Mitsuya et al. 2000). Salinity stress caused swelling of thylakoid as one of the main alterations of chloroplast ultrastructure in barley (Zahra et al. 2014) and rice (Yamane et al. 2012). The wrinkled effects of salinity on chloroplast ultrastructure have been observed at 100 mM NaCl treatment in tomato (Khavari-Nejad and Mostofi 1998) and at 200 mM NaCl treatment potato (Fidalgo et al. 2004) cells under in vitro conditions. The chloroplasts can play a similarly important role as do the mitochondria in triggering PCD, by regulating ROS signaling. The chloroplasts generate more ROS in a less efficient photosynthesis caused by salinity stress, and hence the ROS leads to cell death (Doyle et al. 2010; Kim et al. 2012; Aken and Breusegem 2015; Reape et al. 2015).

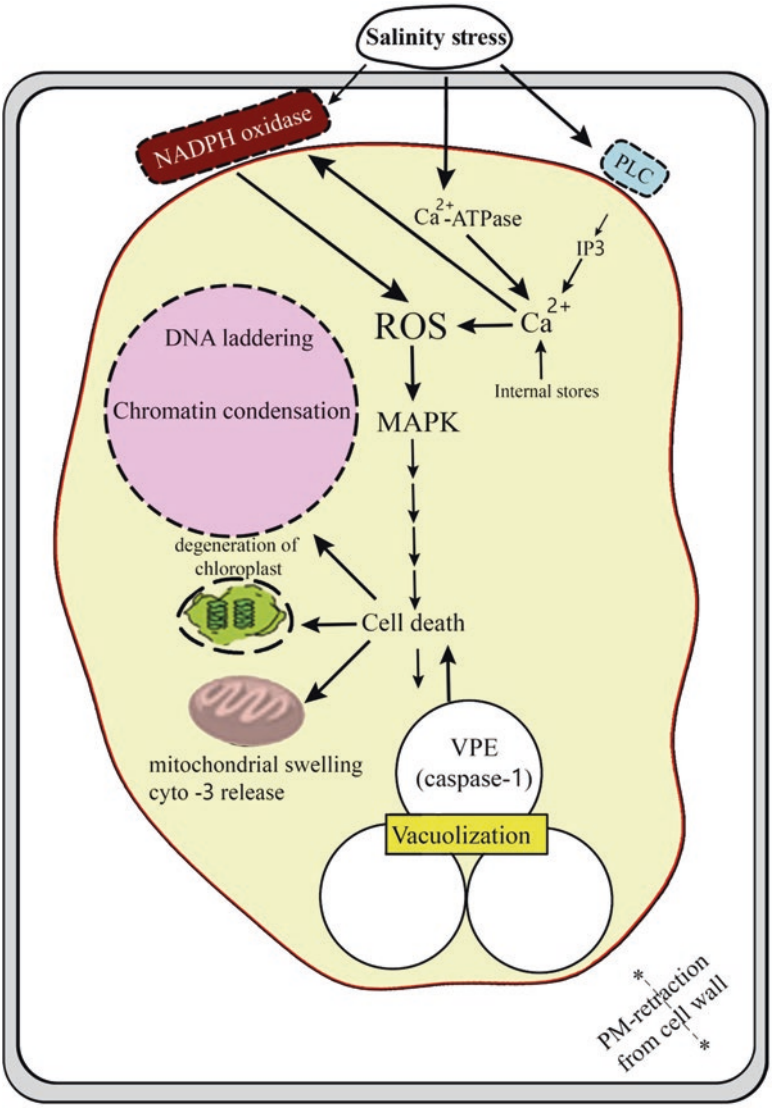


**Fig. 5.2** Overview of salinity stress-induced programmed cell death (PCD) in plants. Salinity stress causes the following changes in the plant cell: (1) phospholipase C (PLC), a plasma membrane (PM) enzyme, liberates IP3 from membrane phospholipid, which results in release of $Ca^{2+}$ from internal stores; (2) an increased $Ca^{2+}$-ATPase gene expression leads to increase in this membrane-bound enzyme, which provides energy to drive the cellular $Ca^{2+}$ pump. An increase $Ca^{2+}$ in cytosol triggers PM-bound NADPH oxidase activity, which produces superoxide in the apoplast. This reactive oxygen species (ROS) transmits death signals through mitogen-activated protein kinases (MAPK) signaling pathway. In addition, death signals can be emitted by vacuolar processing enzyme (VPE) releasing from vacuole. The ROS levels could also be increased by a sense of release of either cytochrome c (Cyt c) or cytochrome f (Cyt f) from mitochondrion. Signals are transmitted to nucleus and ultimately cell execution proteins is synthesized, which results in PCD

A profound downgrading in cytoplasmic streaming was observed at 100 mM NaCl treatment. In plants, cytoplasmic streaming is a marked attribute of cell compartment, in which vesicles and organelles transport along the strands of cytoplasm containing actin filaments. Cytoplasmic streaming indirectly explores some features of the metabolic function in the cell (Mansour and Salama 2004; Shimmen and Yokota 2004; Pieuchot et al. 2015). It has been postulated that salinity stress causes an increase in cytoplasmic $Ca^{2+}$, which may detain cytoplasmic streaming through the support of internal $Ca^{2+}$ stores (Knight 2000). Calcium does not solely play a key role in signaling function but might also trigger PCD and mediate death-specific enzymes in both animal and plant cells (Boursiac et al. 2010). In plants, Zhu et al. (2010) used the RNA-interfering (RNAi) silencing of the $Ca^{2+}$ pump *NbCA1* and showed that endomembrane $Ca^{2+}$ pump operates in mediating the kinetics of a PCD pathway triggered by the pathogen. As shown in Fig. 5.2, a plasma membrane enzyme, phospholipase C (PLC), liberates IP3 from membrane phospholipid. The release of $Ca^{2+}$ from internal stores is in turn mediated by IP3. It is hence suggested that the amplification of the stress signals during stress through enhancing the level of the stress-induced $Ca^{2+}$ signal could be mediated by a stress-induced PLC gene (Hirayama et al. 1995). Likewise, cytosolic $Ca^{2+}$ was increased by salinity stress in tobacco cells after a few minutes of treatment, and membrane potential of mitochondria was diminished before the occurrence of PCD (Lin et al. 2005). Pretreatment of protoplasts with $Ca^{2+}$ chelators such as EGTA or LaCl3 delayed salinity stress-induced PCD through the increase in cytosolic $Ca^{2+}$ implying an essential role for $Ca^{2+}$ in the triggering of PCD in plants (Lin et al. 2005; Li et al. 2007a).

The function of $Ca^{2+}$-ATPases is amended substantially in response to abiotic stress in plants. $Ca^{2+}$-ATPase membrane-bound enzyme hydrolyzes ATP to supply energy to run the cellular $Ca^{2+}$ pump. The transcript levels of genes encoding a putative ER $Ca^{2+}$-ATPase have been increased by salinity stress in tobacco cells (Perez-Prat et al. 1992) and in tomato (Wimmers et al. 1992). In tobacco, Perez-Prat et al. (1992) observed an increased $Ca^{2+}$-ATPase gene expression in both adapted and unadapted cells cultured at 428 mM NaCl, while the levels of transcripts were much higher in adapted cells than in unadapted cells. Likewise, an enhanced $Ca^{2+}$-ATPase transcript has been observed in plants treated with 50 mM NaCl for 24 h in tomato (Wimmers et al. 1992). In addition, it was suggested that the regulation of expression of $Ca^{2+}$-ATPase gene could be regulated by the RNAi such as 22 nt miR4376 in tomato (Wang et al. 2011). It has been demonstrated that $Ca^{2+}$-ATPase comprises five functional domains which are named based on their function or position. Hence, they include P-domain (the catalytic core), A-domain (actuator domain), N-domain (nucleotide-binding domain), as well as S- and T-domains (membrane-embedded domains). The ATP hydrolysis is performed by the cytoplasmic domains (A, P, and N), whereas the T- and S-domains play role in the ion transport, together with conformational changes through tertiary contacts and linkers (Palmgren and Nissen 2011).

It is argued that the burden of PCD categorizations should be put on the tonoplast disruption relating to cytoplasmic clearing since the vacuole elaborates on various plant PCD types including HR, differentiation of tracheary elements,

senescence of various plant tissues, and so on (van Doorn 2011). Accordingly, the only terminology of two classes of PCD comprising necrosis and vacuolar cell death was suggested by van Doorn (2011). Vacuolar cell death is caused by a progression of an autophagy-associated phenomenon and the release of hydrolases from ruptured vacuoles (Bagniewska-Zadworna and Arasimowicz-Jelonek 2016). In addition, vacuole disintegration and tonoplast disruption are extremely rapid and irreversible processes and represent an unequivocal step in a cell headed for death in plant roots (Bagniewska-Zadworna and Arasimowicz-Jelonek 2016). In *Physcomitrella patens*, it was shown that knockout of vacuolar ACA pump (*PCA1*) gene could lead to higher sensitivity to salinity stress, because of diminished level of NaCl-triggered $Ca^{2+}$ in the cytosol (Qudeimat et al. 2008). Furthermore, the rapid enlargement of vacuolar volume has been observed under in vitro salinity conditions in mangrove [*Bruguiera sexangula* (Lour.) Poir.] cells and barley (*Hordeum vulgare* L. cv. Doriru) root meristematic cells (Mimura et al. 2003). Paradoxically, this phenomenon was not confirmed in pea (*Pisum sativum* L.) (Mimura et al. 2003). The accumulation of $Na^+$ ions in the central vacuole causes enhanced vacuolar volumes and is considered as one of the strategies employed by the cell in response to salinity stress.

The detrimental effects of ROS on plant tissues are being increasingly recognized, but the biochemical mechanism linking the ROS production and PCD is poorly known. The main enzymes responsible for superoxide anion generation are cell wall-associated peroxidases and apoplastic plasma membrane-bound NADPH oxidases which are regulated by various environmental and developmental stimuli (Gechev et al. 2006; Sagi and Fluhr 2006). Salinity stress causes an increase in $Ca^{2+}$ in the cytosol and triggers PM-bound NADPH oxidase activity, which produces superoxide in the apoplast (Monetti et al. 2014). Nevertheless, ROS are involved in signaling pathways and mediating PCD activation (Chen et al. 2009a; Mittler 2017) as they influence the activity of mitogen-activated protein kinase (MAPK), which is able to induce several nuclear transcription factors (Fig. 5.2). The overaccumulation of ROS in the cells causes oxidative cellular damage and cell death through reacting with different cellular components. In addition, it is now appreciated that ROS involves in triggering a programmed or physiological pathway for cell death that were not previously thought to be associated straightforwardly with executing cells through oxidation (Mittler 2017). In general, the quantity of ROS accumulation can activate opposing pathways leading to either survival or PCD. A supportive data was obtained by *Chlamydomonas reinhardtii* subjected to hydrogen peroxide ($H_2O_2$) treatment. The programmed cell suicide event is shown to be triggered by enhanced level of $H_2O_2$ which resulted in caspase-3-like protein recruitment, DNA laddering, and increased cleavage of PARP (a poly-(ADP)-ribose polymerase-like enzyme) (Vavilala et al. 2015). In general, a variety of genes, transcription factors, and signaling molecules associated with the inducible expression of genes mediating salinity-induced PCD. Certainly, this series includes some of those appointed to control ROS accumulation, release of Cyt c and $Ca^{2+}$, and mitochondrial permeability transition (Lin et al. 2005, 2006; Li et al. 2007b; Chen et al. 2009a; Monetti et al. 2014; Biswas and Mano 2015; Bahieldin et al. 2016; Pan et al. 2016).

PM and its proteins involved in a wide spectrum of cellular processes including signal perception-transduction and cellular homeostasis, which are regulated by various developmental and environmental stimuli (Mansour 2014; Mansour et al. 2015). Salinity stress-induced PCD has also associated with the retraction of the PM from the cell wall, most likely due to raising the osmotic pressure leading to plasmolysis (Dauphinee et al. 2014; Zhang et al. 2016). Salinity stress is also known to cause membrane disturbance resulting in the loss of membrane integrity, which allows intracellular components to leak out of the cells. NaCl-induced $K^+$ efflux is believed to be responsible for the effect of salinity on the loss of membrane integrity and non-specific membrane damage in a number of species (Shabala et al. 2006; Cuin et al. 2008; Demidchik et al. 2010, 2014).

The cell membrane is the first living tissue that perceives signals of abiotic stresses including salinity and because of important role and abundance of lipids, which is one of the most sensitive ROS targets (Mansour 2014; Mansour et al. 2015). Salinity stress may cause electrolyte leakage as it is one of the integral parts of the plant's response to stress. Demidchik et al. (2014) suggested that the main consequence of electrolyte leakage is stress-induced $K^+$ release which outwardly rectifying $K^+$ channels activated by ROS are responsible for this in plant cells. The $K^+$ loss results from ion channel-mediated $K^+$ efflux can induce PCD (Demidchik et al. 2010, 2014). The phenomenon of ROS generation, leading also to PCD, is not an independent process but may largely be influenced by the $K^+$ loss in conditions of stress-induced electrolyte leakage. In plant cells, highly selective outward-rectifying potassium channel (SKOR), guard cell outward-rectifying K+ channel (GORK), and annexins catalyzing $K^+$ efflux can be activated by ROS (•OH and $H_2O_2$). In addition, under salinity and oxidative stress, PCD could be induced by GORK-mediated $K^+$ efflux (Demidchik et al. 2014).

The mitochondrion has recently acquired renewed attention in toxicology because of its crucial role in signaling and mediating cell death in certain cell types. It was proposed that mitochondria can also be associated in signaling pathways relevant to PCD induction, which is the mitochondrial release of cytochrome c and $Ca^{2+}$ into the cytosol where they trigger cell death caspases (Lin et al. 2005, 2006; Reape et al. 2015). In plants, the permeability of the mitochondrial membrane increases due to mitochondrial generated ROS which releases apoptotic mediators such as cytochrome c (see Fig. 5.2; Tiwari et al. 2002). Salinity stress caused partially or fully inactivation of the photosynthetic reaction centers which results in the downgraded conversion of light energy into chemical energy (Turan and Tripathy 2015), leading to increased ROS formation (Ambastha et al. 2017). In rice, a recent study of the ultrastructure of seedling leaves has proposed the involvement of chloroplasts in PCD induced by salinity stress (Ambastha et al. 2017). Salinity stress also reported inducing cell death in isolated protoplasts of tobacco (Lin et al. 2006) and rice (Ambastha et al. 2017).

Salinity stress may cause substantial amendments in the Golgi bodies and disordered vesicle formation and trafficking in plant cells. In *Arabidopsis thaliana*, high NaCl levels promoted vesicle formation, which may imply elevated levels of macro-

autophagy, plausibly to recycle degenerated intracellular elements (Liu et al. 2009). The ultrastructural alterations were observed in not only mitochondria but also Golgi bodies, which eventually resulted in autophagy in a halophyte plant, *Thellungiella halophila* (Wang et al. 2010).

## 5.4 Types of NaCl-Induced Cell Death in Plants

The first approach taken by plant cells for apoptosis-like PCD through DNA fragmentation does not include the NaCl-induced osmotic stress because osmotic stress cannot be accounted for the activation of endonucleases. DNA laddering results in PCD were only found in cells with NaCl and KCl treatment and not in sorbitol-treated cells, indicating that ionic component is to be associated with the PCD (Affenzeller et al. 2009; Vavilala et al. 2016). Hence, the effects of $Na^+$ ion toxicity have attracted much greater interest as an apt target for dissecting the PCD and the salinity tolerance mechanisms than the osmotic effects (Arzani and Ashraf 2016). Potassium serves as a macronutrient with important roles in a variety of physiological processes in plants, including nucleic acid and protein synthesis. The second approach taken for NaCl-induced PCD is related to ion homeostasis disturbance that results from an excessive amount of $Na^+$ and a $K^+$ deficit in the cytosol. It is postulated that reduction of cytosol $K^+/Na^+$ ratio in the cells would be an essential component in triggering PCD (Joseph and Jini 2010). Under saline conditions, the influx of $Na^+$ through plasma membrane by the nonselective cation channels (NSCC) causes plasma membrane depolarization which leads to $K^+$ leakage from the cell through depolarization-activated potassium outward-rectifying channels (KORs) (Shabala 2009; Demidchik 2014; Kim et al. 2014). $K^+$ deficit results from the release of $K^+$ from the cytoplasm, which in turn may trigger the effectors of PCD, cysteine proteases (Shabala 2009; Demidchik et al. 2010). The final way taken for the PCD is associated with NaCl-induced oxidative stress, generating ROS, which causes PCD through the deleterious effects to nucleic acids, proteins, lipids, and enzymes, as well as increased peroxidation of membrane lipids and membrane leakage. The enhanced ROS and reduced mitochondrial membrane potential were observed in protoplasts of *Nicotiana tabacum* treated with salinity stress. Similarly, increase in cytosolic $Ca^{2+}$ was found a few minutes after salinity treatment, and decreased membrane potential of mitochondria was also noticed before the occurrence of PCD in tobacco BY2 cells (Monetti et al. 2014). In *Thellungiella halophila*, salinity stress-induced PCD through caspase-like proteases under in vitro conditions was observed. Cells undergoing PCD exhibited attributes such as DNA laddering, retraction of plasma membrane from the cell wall, Cyt c release, and increase in caspase-3-like protease activity (Wang et al. 2010). The cells subjected to in vitro salt stress (500 mM NaCl) showed PCD symptoms such as DNA laddering, nuclear condensation, reduced cell viability, and positive TUNEL in wheat (Rezaei et al. 2013).

## 5.5 Engineering PCD Pathway to Enhance Salinity Tolerance

Transgenic plants regenerated from the cells transformed with recombinant DNA are becoming increasingly pervasive and will approach ubiquity in research laboratories. The production of transgenic plants has become commonplace and has been employed as a routine tool for the introduction of a foreign or related gene to an agronomically important crop variety and for elucidating mechanisms of gene expression. Transgenic plants expressing novel salinity tolerance genes can be employed to improve crop performance under saline conditions (Arzani and Ashraf 2016). The prosperous development of transgenic plants with the desired trait, such as salinity tolerance, relies on object identification of the genes that are key players in governing that trait. Although overexpression of the majority of salinity tolerance genes being in model plants such as tobacco or *Arabidopsis* plants, the list of candidate genes mainly associated with $Na^+$ exclusion in the transgenic plants from both *Arabidopsis* and field crops has been compiled by Arzani and Ashraf (2016). The various strategies to engineer PCD pathways that enhance salinity tolerance are as follows:

### *5.5.1 Manipulation of Anti-PCD Genes*

The development of transformed plants for resistance to apoptosis could be an effective approach to improving salinity tolerance. It has been revealed that the generation of transgenic plants expressing anti-PCD genes led to enhancing biotic and abiotic tolerance. The family of apoptosis-stimulating proteins of p53 (ASPP) with iASPP, as the most evolutionary conserved member (Sullivan and Lu 2007), is one of the most promising candidates for use as anti-apoptotic factors. The ASSP family members bind to key player proteins regulating cell growth (APCL, PP1) and apoptosis (p53, p63, p73, Bcl-2, and RelA/p65) and most likely regulate the apoptotic function of p53, p63, and p73 (Sullivan and Lu 2007). The iASPP proteins only inhibit the apoptotic function of P53 (including p63 and p73) and do not impact the cell-cycle arrest activity of p53.

The expression of different apoptotic Bcl-2 genes can be activated by p53 as a transcription factor (Levine and Oren 2009).

In mammals, the family of Bcl-2 (B-cell lymphoma2) proteins, localized in the outer mitochondrial membrane, is a key regulator of mitochondrial outer membrane permeabilization (MOMP) and subsequent apoptosis. Bcl-2 proteins comprise both anti-apoptotic member (Bcl-2, Bcl-XL, and Mcl-1) proteins and the pro-apoptotic (Bax, Bak, and Bad) members (Le Pen et al. 2016). They exert influence on balancing the mitochondrial membrane potential. Although the members of Bcl-2 family, caspases, and the members of the inhibitor of apoptosis (IAP) family are important regulators of apoptosis in animals, conservation cycle does not evidently occur in plants. However, plant PCD and animal apoptosis have many common morphological resemblances. The expression of anti-apoptotic (pro-survival) genes has generally

been investigated in the model or crop plants using animal and plant target genes. For example, tomato plants were transformed with animal anti-apoptotic *Bcl-xL* and *Ced-9* genes and led to retarded cell death or lack of cucumber mosaic virus symptoms (Xu et al. 2004). Tomato and tobacco plants expressing *SfIAP* gene from an insect (*Spodoptera frugiperda*) preclude cell death caused by the necrotrophic fungus *Alternaria alternata*, salinity, heat, and fungal toxin fumonisin B1 (FB1) treatment (Li et al. 2010). Likewise, expression of *SfIAP* gene has enhanced salinity tolerance in rice (Hoang et al. 2014).

The family of Bcl-2-associated athanogene (BAG) proteins is conserved in the eukaryotic organisms. The anti-cell death activity of BAG has been described through constitutive overexpression of *AtBAG4* in rice (Hoang et al. 2015). All BAG proteins share a common signature motif at the C terminus (BD domain), which directly mediates binding to the Hsp70/Hsc70 heat shock proteins (see the recent review by Kabbage et al. 2017). The ubiquitous 70 kDa Hsp70 family proteins play a crucial role, as molecular chaperones in mediating the refolding of denatured proteins and the folding of newly synthesized proteins. Therefore, Hsp70 proteins can assist anti-apoptotic Bcl-2 proteins through protein-protein interaction at marked essential points to suppress apoptosis pathways (Joly et al. 2010). Overexpression of *Hsp70* derived from *Citrus tristeza* virus in rice conferred tolerance to salinity stress (Hoang et al. 2015). In rice, transgenic plants overexpressing *Bcl-2* gene significantly alleviated PCD symptoms through reduction of NaCl-induced $K^+$ efflux and inhibition of the expression of VPEs (Kim et al. 2014).

### 5.5.2 Overexpression of Inhibitor of Apoptosis (IAP) Genes

Although plant genomes do not contain IAPs, tolerance to cell death induced by stress has been detected in the ectopic expression of viral and animal IAPs in plants. In tobacco, transgenic plants overexpressing the baculovirus *Orgyia pseudotsugata* nuclear polyhedrosis virus IAP (OpIAP) protein were resistant to tomato-spotted wilt virus and the necrotrophic fungi *Cercospora nicotianae* and *Sclerotinia sclerotiorum* (Dickman et al. 2001).

### 5.5.3 Interfering RNA (RNAi)-Induced Apoptosis Gene Silencing

Alternatively, small interfering RNA (siRNA)-induced transcriptional gene silencing system can be used to knockdown or knockout the expression of apoptotic genes. Long noncoding (lncRNA) and microRNAs miRNA are the two foremost subtypes of regulatory noncoding RNA (ncRNAs). They comprehensively regulate the interrelated steps and mediate the regulated cell death including apoptosis and necrosis through their interaction as well as in association with assorted

intracellular components (Su et al. 2016). Cytoplasmic mRNAs can be silenced by miRNAs through either promoting translation repression, expediting mRNA decapping, or triggering an endonuclease cleavage (Bagga et al. 2005; Wu et al. 2006; Pasquinelli 2012; Nam et al. 2014). As such, the alternative cleavage and polyadenylation mechanisms that produce varied 3′-UTR isoforms influence the efficiency of miRNA targeting, while the translation inhibition is dependent on the CCR4-NOT complex and the miRNA-induced silencing complex (miRISC), which causes the recruitment of eIF4A2 and locked on the mRNA region between the start codon and the pre-initiation complex (Nam et al. 2014). In humans, loss of microRNA-mediated repression of *Bcl2* gene expression, in many instances, causes chronic lymphocytic leukemia (CLL) (Anderson et al. 2016). RNAi-mediated silencing of *P69B* a substrate of two matrix metalloproteinases (Sl2/3-MMP) from tomato and located upstream of *Sl2/3-MMP* in tomato transgenic plants led to reduced expression of the cell death marker genes *tpoxC1*, *hsr203j*, and *Hin1* (Zimmermann et al. 2016). VPEs are cysteine proteinases that function as key moderators of stress-induced PCD in plants. Suppression of *OsVPE3* gene in the transgenic lines of rice led to improved salinity tolerance (Lu et al. 2016). Transgenic rice plants overexpressing Bcl-2 resulted in inhibition of salt-induced PCD through a significant reduction of the transient increase in the cytosolic $Ca^{2+}$, suppression of *OsVPE2* and *OsVPE3*, expression, and inhibited $K^+$ efflux across the plasma membrane (Kim et al. 2014). In *Arabidopsis*, inhibition of FB1-induced cell death was observed using loss of function mutation in all four VPE (αVPE, βVPE, γVPE, and δVPE) genes (Kuroyanagi et al. 2005). In *Nicotiana benthamiana*, silencing $VPE_{1a}$ and $VPE_{1b}$ diminished sensitivity to cell death caused by the elicitor of bacterial hairpin but did not affect cell death caused by ethylene-inducing peptide1 (Nep1), the fungal necrosis, and the elicitor of oomycete boehmerin (Zhang et al. 2010). Therefore, although $VPE_{1a}$ and $VPE_{1b}$ may involve in elicitor-triggered immunity, they execute cell death in a context-specific manner.

### *5.5.4 Repression of ROS-Induced PCD*

The signaling and biological roles of ROS (e.g., $^{\bullet}O^{-}_{2}$, $H_2O_2$, $^{\bullet}OH$, $^{1}O_2$) in higher-order eukaryotic cells are still controversial and are unclear. Paradoxically, it is conceivable that both the stimulatory and inhibitory capacities of ROS can be related to its conspicuous biological properties, which comprise half-life, chemical reactivity, and lipid solubility (D'Autreaux and Toledano 2007). ROS, on the one hand, appear to act as signaling molecules that mediate intercellular pathways controlling cell growth, differentiation, inflammation, survival, and immunity when available at a moderate levels (D'Autreaux and Toledano 2007; Foyer and Noctor 2016; Gilroy et al. 2016; Mittler 2017). On the other hand, the excessive generation of ROS results in oxidative damage to essential biological molecules such as DNA, RNA, membranes (lipid peroxidation), and proteins, which causes the demolition of cellular integrity through amending their functionality. During normal homeostasis,

endogenous ROS production mainly takes place in the Ero1-PDI oxidative folding system in ER, the electron transport chain in the mitochondrion, and the membrane-bound NADPH oxidase (NOX) complex (Sevier and Kaiser 2008). Considering PCD can be attained by enhanced ROS accumulation and abiotic conditions like salinity stress, genetic programming of cellular metabolism in plants, repressing salinity stress-induced PCD, would lead to an equal relative increase in yield under saline conditions (Xu et al. 2004; Mittler and Blumwald 2010; Hoang et al. 2015). Constitutive overexpression of maize *ABP9* (ABRE-binding protein 9) gene in transgenic *Arabidopsis* plants downregulated cellular ROS content induced by stress and ABA and diminishes cell death (Zhang et al. 2011). Interestingly, aside from the key roles of $SOS_1$ and $SOS_2$ in salinity tolerance (see the recent review by Arzani and Ashraf 2016) under salinity stress conditions, they influence the expression of other genes involving in the ROS scavenging activity. Verslues et al. (2007) reported the physical interaction between $SOS_2$ and $NDPK_2$ ($H_2O_2$ signaling protein) with CATs. Expression of a baculovirus anti-apoptotic protein, p35, has been observed to suppress PCD induced by $H_2O_2$ in insect cells through clearly sequestering ROS. It was speculated that the ROS contents can be regulated by either *p35* gene directly or *Hsp70* and *AtBAG4* genes indirectly. In tobacco, transgenic plants expressing p35 (gene from *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV)) enhanced abiotic stress tolerance including salinity, which was associated with the capacity to scavenge ROS by p35 (Wang et al. 2009). As a final overview, Table 5.1 summarizes the reported candidate genes involving in PCD pathway and while overexpressed in the transgenic plants to enhance salinity tolerance.

## 5.6 Concluding Remark

The molecular mechanisms of salinity-induced PCD via autophagy cell death (ACD) remain to be elucidated by studying the autophagic vacuolization of the cytoplasm and the dynamics of the vacuole in various plant species. Apoptosis and anti-apoptosis phenomena occur as a consequence of the successive development of genetic alterations in multiple genes and epigenetic changes that regulate activities of apoptotic caspases responsible for the execution of various PCD. Therefore, another area of research which illuminates these phenomena is that which explores DNA modifications and dynamic histones related to crucial alterations of genome expression during the PCD. Hence, studies to elucidate the common and innovation features existing between abiotic-induced PCD and pathogen-induced PCD will assist in understanding the physicochemical details of apoptotic-like PCD which needs for selectively manipulating target cell in each of the two conditions.

Nevertheless, improving salinity tolerance through manipulation of PCD pathways in crop plants could be attained by:

1. Upregulation/overexpression of anti-apoptosis genes and downregulation or suppression of pro-apoptosis genes which are functionally indispensable and structurally conserved throughout the plant and animal kingdoms. For instance, the

**Table 5.1** Candidate genes involving in programmed cell death (PCD) pathway overexpressed[a] in the transgenic plants to enhance salinity tolerance

| Gene | Function | Origin species | Transgenic plant species | Effects on PCD/mechanism | References |
|---|---|---|---|---|---|
| *Ced-9* homolog of *Bcl-2* | Anti-apoptotic | *Caenorhabditis elegans* | *Nicotiana benthamiana* | Enhanced tolerance to salinity and oxidative stress by altering $H^+$ and $K^+$ flux hypothetically by $K^+$-permeable channels (KOR and NSCC) | Shabala et al. (2007) |
| *SOS1* salt overly sensitive 1 | Efflux of $Na^+$ from cells | *Homo sapiens* | *Arabidopsis thaliana* | Arabidopsis *sos1* mutants displayed higher PCD symptoms, showing salinity-induced PCD is regulated by ion disequilibrium | Huh et al. (2002) |
| *Bcl-2* B-cell lymphoma | Anti-apoptotic | *Homo sapiens* | *Oryza sativa* | Suppressed $K^+$ efflux across the PM by blocking NSCCs, reduction of cytoplasmic $Ca^{2+}$, and inhibited the expression of *OsVPE2* and *OsVPE3*, leading to the alleviated salinity-induced PCD | Deng et al. (2011) |
| *Bcl-2* | Anti-apoptotic | *Homo sapiens* | *Oryza sativa* | Alleviated PCD symptoms through reduction of NaCl-induced K+ efflux, inhibition of the expression of VPEs | Kim et al. (2014) |
| *Hsp70* heat shock protein | Assist anti-apoptotic *Bcl-2* | *Citrus tristeza* virus | *Oryza sativa* | Enhanced salinity tolerance via alleviation of PCD | Hoang et al. (2015) |
| *IAP* | Inhibitor of apoptosis | *Spodoptera frugiperda* (*SfIAP*) | *Oryza sativa* | Enhanced salinity tolerance via precluding PCD | Hoang et al. (2014) |
| *BAG* | Bcl-2-associated athanogene | *A. thaliana* (*AtBAG4*) | *Oryza sativa* | Enhanced salinity tolerance via precluding PCD | Hoang et al. (2015) |
| *VPE3* RNAi suppressed | Vacuolar processing enzymes (VPEs) | *Oryza sativa* (*OsVPE3*) | *Oryza sativa* | Enhanced salinity tolerance with downregulated *OsVPE3* | Lu et al. (2016) |

| Gene | Function | Origin species | Transgenic plant species | Effects on PCD/mechanism | References |
|---|---|---|---|---|---|
| *p35* | Anti-apoptotic | Baculovirus (*Autographa californica* multiple nucleopolyhedrovirus (AcMNPV)) | *Nicotiana tabacum* | Enhanced salinity tolerance with ability to scavenge ROS | Wang et al. (2009) |
| *p35* | Anti-apoptotic | Same as above | *Oryza sativa* | Enhanced salinity tolerance with ability to scavenge ROS | Hoang et al. (2015) |
| *MKK4* | A MAPK kinase | (*GhMKK5*) | *Nicotiana benthamiana* | Reduced salinity tolerance via increase in $H_2O_2$-induced HR-like PCD | Zhang et al. (2012) |
| *HSPR* | Heat shock protein related | *A. thaliana* (*AtHSPR*) | *A. thaliana* | Protect cells from death upon salinity stress | Yang et al. (2015) |
| *OsSRP-LRS* RNAi suppressed; *AtSerpin1* homolog | Serine protease inhibitors (serpins) | *Oryza sativa* | *Oryza sativa* | Negatively regulates stress-induced cell death | Bhattacharjee et al. (2015) |

[a]Unless otherwise stated

protein members of the Bcl-2 family comprised both pro-apoptosis and anti-apoptosis genes that regulate the release of cytochrome c and other apoptotic alterations in the mitochondrion.
2. The repression of the plant caspase-like enzymes including VPEs, metacaspases, and phytaspases also called subtilisin-like proteases (subtilases) are alternative candidates for "silencing" or "downregulation" by emerging genetic and epigenetic tools.

## References

Affenzeller MJ, Darehshouri A, Andosch A, Lu C, Lütz-Meindl U (2009) Salt stress-induced cell death in the unicellular green alga *Micrasterias denticulata*. J Exp Bot 60:939–954

Aken O, Breusegem F (2015) Licensed to kill: mitochondria, chloroplasts, and cell death. Trends Plant Sci 20:754–766

Ambastha V, Sopory SK, Tiwari BS, Tripathy BC (2017) Photo-modulation of programmed cell death in rice leaves triggered by salinity. Apoptosis 22:41–56

Anderson MA, Deng J, Seymour JF, Tam C, Kim SY, Fein J, Yu L, Brown JR, Westerman D, Si EG, Majewski IJ, Segal D, Enschede SLH, Huang DCS, Davids MS, Letai A, Roberts AW (2016) The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. Blood 127:3215–3224

Ariño-Estrada G, Mitchell GS, Saha P, Arzani A, Cherry SR, Blumwald E, Kyme AZ (2017) Imaging salt transport in plants using PET: a feasibility study. IEEE nuclear science symposium and medical imaging conference 2017 (IEEE NSS/MIC 2017)

Arzani A (2008) Improving salinity tolerance in crop plants: a biotechnological view. In Vitro Cell Dev Biol Plant 44:373–383

Arzani A, Ashraf M (2016) Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Crit Rev Plant Sci 35:146–189

Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122:553–563

Bagniewska-Zadworna A, Arasimowicz-Jelonek M (2016) The mystery of underground death: cell death in roots during ontogeny and in response to environmental factors. Plant Biol 18:171–184

Bahieldin A, Atef A, Edris S, Gadalla NO, Ali HM, Hassan SM, Al-Kordy MA, Ramadan AM, Makki RM, Al-Hajar ASM, El-Domyati FM (2016) Ethylene responsive transcription factor ERF109 retards PCD and improves salt tolerance in plant. BMC Plant Biol 16:216. https://doi.org/10.1186/s12870-016-0908-z

Balk J, Leaver CJ, McCabe P (1999) Translocation of cytochrome c from the mitochondria to the cytosol occurs during heat-induced programmed cell death in cucumber plants. FEBS Lett 463:151–154

Bhattacharjee L, Singh PK, Singh S, Nandi AK (2015) Down-regulation of rice serpin gene *OsSRP-LRS* exaggerates stress-induced cell death. J Plant Biol 58:327–332

Bialik S, Zalckvar E, Ber Y, Rubinstein AD, Kimchi A (2010) Systems biology analysis of programmed cell death. Trends Biochem Sci 35:556–564

Biswas MS, Mano J (2015) Lipid peroxide-derived short-chain carbonyls mediate hydrogen peroxide-induced and salt-induced programmed cell death in plants. Plant Physiol 168:885–898

Bonneau L, Ge Y, Drury GE, Gallois P (2008) What happened to plant caspases? J Exp Bot 59:491–499

Boursiac Y, Lee SN, Romanowsky S, Blank R, Sladek C, Chung WS, Harper JF (2010) Disruption of the vacuolar calcium-ATPases in Arabidopsis results in the activation of a salicylic acid-dependent programmed cell death. Plant Physiol 154:1158–1171

Cande C, Cecconi F, Dessen P, Kroemer G (2002) Apoptosis-inducing factor (AIF): key to the conserved caspase-independent pathways of cell death? J Cell Sci 115:4727–4734

Chen R, Sun S, Wang C, Li Y, Liang Y, An F, Li C, Dong H, Yang X, Zhang J, Zuo J (2009a) The Arabidopsis PARAQUAT RESISTANT2 gene encodes an S-nitrosoglutathione reductase that is a key regulator of cell death. Cell Res 19:1377–1387

Chen X, Wang Y, Li J, Jiang A, Cheng Y, Zhang W (2009b) Mitochondrial proteome during salt stress-induced programmed cell death in rice. Plant Physiol Biochem 47:407–415

Conde A, Chaves MM, Geros H (2011) Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiol 52:1583–1602

Cuin TA, Betts SA, Chalmandrier R, Shabala S (2008) A root's ability to retain $K^+$ correlates with salt tolerance in wheat. J Exp Bot 59:2697–2706

D'Autreaux B, Toledano MB (2007) ROS as signaling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8:813–824

Dauphinee AN, Warner S, Gunawardena AH (2014) A comparison of induced and developmental cell death morphologies in lace plant (*Aponogeton madagascariensis* ) leaves. BMC Plant Biol 14:389

De Pinto MC, Locato V, De Gara L (2012) Redox regulation in plant programmed cell death. Plant Cell Environ 35:234–244

Demidchik V (2014) Mechanisms and physiological roles of $K^+$ efflux from root cells. J Plant Physiol 171:696–707

Demidchik V, Cuin TA, Svistunenko D, Smith SJ, Miller AJ, Shabala S, Sokolik A, Yurin V (2010) Arabidopsis root $K^+$-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. J Cell Sci 123:1468–1479

Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V (2014) Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. J Exp Bot 65:1259–1270

Deng M, Bian H, Xie Y, Kim Y, Wang W, Lin E, Zeng Z, Guo F, Pan J, Han N, Wang J, Qian Q, Zhu M (2011) Bcl-2 suppresses hydrogen peroxide-induced programmed cell death via OsVPE2 and OsVPE3, but not via OsVPE1 and OsVPE4, in rice. FEBS J 278:4797–4810

Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R (2001) Abrogation of disease development in plants expressing animal antiapoptotic genes. Proc Natl Acad Sci U S A 98:6957–6962

Doyle SM, Diamond M, McCabe PF (2010) Chloroplast and reactive oxygen species involvement in apoptotic-like programmed cell death in Arabidopsis suspension cultures. J Exp Bot 61:473–482

Fidalgo F, Santos A, Santos I, Salema R (2004) Effects of long-term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants. Ann Appl Biol 145:185–192

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875

Foyer CH, Noctor G (2016) Stress-triggered redox signaling: what's in pROSpect? Plant Cell Environ 39:951–964

Gechev TS, Van Breusegem F, Stone JM, Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28:1091–1101

Gilroy S, Białasek M, Suzuki N, Górecka M, Devireddy A, Karpinski S, Mittler R (2016) ROS, calcium and electric signals: key mediators of rapid systemic signaling in plants. Plant Physiol 171:1606–1615

Green DR (2011) Means to an end. Apoptosis and other cell death mechanisms. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149–190

Hamed-laouti IB, Arbelet-bonnin D, De Bont L, Biligui B, Gakière B, Abdelly C, Ben Hamed K (2016) Comparison of NaCl-induced programmed cell death in the obligate halophyte *Cakile maritima* and the glycophyte *Arabidopsis thaliana*. Plant Sci 247:49–59

Hatsugai N, Yamada K, Goto-Yamada S, Hara-Nishimura I (2015) Vacuolar processing enzyme in plant programmed cell death. Front Plant Sci 6:234
Hernandez JA, Olmos E, Corpas FJ, Sevilla F, del Rio LA (1995) Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci 105:151–167
Hirayama T, Ohto C, Mizoguchi T, Shinozaki K (1995) A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. Proc Nat Acad Sci USA 92:3903–3907
Hoang TML, Williams B, Khanna H, Dale J, Mundree SG (2014) Physiological basis of salt stress tolerance in rice expressing the anti-apoptotic gene *SfIAP*. Funct Plant Biol 41:1168–1177
Hoang TML, Moghaddam L, Williams B, Khanna H, Dale J, Mundree SG (2015) Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. Front Plant Sci 6:175
Hogg B, Kacprzyk J, Molony EM, O'Reilly C, Gallagher TF, Gallois P (2011) An in vivo root hair assay for determining rates of apoptotic-like programmed cell death in plants. Plant Methods 7:45
Huett A, Goel G, Xavier RJ (2010) A systems biology viewpoint on autophagy in health and disease. Curr Opin Gastroenterol 26:302–309
Huh G, Damsz B, Matsumoto TK, Reddy MP, Rus AM, Ibeas JI, Narasimhan ML, Bressan RA, Hasegawa PM (2002) Salt causes ion disequilibrium-induced programmed cell death in yeast and plants. Plant J 29:649–659
Huysmans M, Lema AS, Coll NS, Nowack MK (2017) Dying two deaths—programmed cell death regulation in development and disease. Curr Opin Plant Biol 35:37–44
Joly A, Wettstein G, Mignot G, Ghiringhelli F, Garrido C (2010) Dual role of heat shock proteins as regulators of apoptosis and innate immunity. J Innate Immun 2:238–247
Joseph B, Jini D (2010) Salinity induced programmed cell death in plants: challenges and opportunities for salt-tolerant plants. J Plant Sci 5:376–390
Kabbage M, Kessens R, Bartholomay LC, Williams B (2017) The life and death of a plant cell. Annu Rev Plant Biol 68:1–7. https://doi.org/10.1146/annurev-arplant-043015-111655
Kerr JFR, Wylie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implication in tissue kinetics. Br J Cancer 26:239–257
Khavari-Nejad RA, Mostofi Y (1998) Effects of NaCl on photosynthetic pigments, saccharides, and chloroplast ultrastructure in leaves of tomato cultivars. Photosynthetica 35:151–154
Kim C, Meskauskiene R, Zhang S, Lee K, Ashok M, Blajecka K, Herrfurth C, Feussner I, Apela K (2012) Chloroplasts of Arabidopsis are the source and a primary target of a plant-specific programmed cell death signaling pathway. Plant Cell 24:3026–3039
Kim Y, Wang M, Bai Y, Zeng Z, Guo F, Han N, Bian H, Wang J, Pan J, Zhu M (2014) Bcl-2 suppresses activation of VPEs by inhibiting cytosolic $Ca^{2+}$ level with elevated $K^+$ efflux in NaCl-induced PCD in rice. Plant Physiol Biochem 80:168–175
Knight H (2000) Calcium signaling during abiotic stress in plants. Int Rev Cytol 195:269–325
Koukalova B, Kovarik A, Fajkus J, Siroky J (1997) Chromatin fragmentation associated with apoptotic changes in tobacco cells exposed to cold stress. FEBS Lett 414:289–292
Kroemer G, Martin SJ (2005) Caspase-independent cell death. Nat Med 11:725–730
Kumar SR, Mohanapriya G, Sathishkumar R (2016) Abiotic stress-induced redox changes and programmed cell death in plants—a path to survival or death? In: Gupta DK, Palma JM, Corpas FJ (eds) Redox state as a central regulator of plant-cell stress responses. Springer, Germany, pp 233–252
Kuroyanagi M, Yamada K, Hatsugai N, Kondo M, Nishimura M, Hara-Nishimura I (2005) Vacuolar processing enzyme is essential for mycotoxin-induced cell death in *Arabidopsis thaliana*. J Biol Chem 280:32914–32920
Lam E (2004) Controlled cell death, plant survival and development. Nat Rev Mol Cell Biol 5:305–315
Lam E, Pontier D, del Pozo O (1999) Die and let live: programmed cell death in plants. Curr Opin Plant Biol 2:502–507

Le Pen J, Laurent M, Sarosiek K, Vuillier C, Gautier F, Montessuit S, Martinou JC, Letaï A, Braun F, Juin PP (2016) Constitutive p53 heightens mitochondrial apoptotic priming and favors cell death induction by BH3 mimetic inhibitors of BCL-xL. Cell Death Dis 7:e2083

Levine AJ, Oren M (2009) The first 30 years of p53: growing ever more complex. Nat Rev Cancer 9:749–758

Li J, Jiang A, Chen H, Wang Y, Zhang WPB (2007a) Lanthanum prevents salt stress-induced programmed cell death in rice root tip cells by controlling early induction events. J Integr Biol 49:1024–1031

Li J, Jiang A, Zhang W (2007b) Salt stress-induced programmed cell death in rice root tip cells. J Integr Plant Biol 49:481–486

Li W, Kabbage M, Dickman MB (2010) Transgenic expression of an insect inhibitor of apoptosis gene, *SfIAP*, confers abiotic and biotic stress tolerance and delays tomato fruit ripening. Physiol Mol Plant Pathol 74:363–375

Lin J, Wang Y, Wang G (2005) Salt stress-induced programmed cell death via $Ca^{2+}$-mediated mitochondrial permeability transition in tobacco protoplasts. Plant Growth Regul 45:243–250

Lin J, Wang Y, Wang G (2006) Salt stress-induced programmed cell death in tobacco protoplasts is mediated by reactive oxygen species and mitochondrial permeability transition pore status. J Plant Physiol 163:731–739

Lisenbee CS, Lingard MJ, Trelease RN (2005) Arabidopsis peroxisomes possess functionally redundant membrane and matrix isoforms of monodehydroascorbate reductase. Plant J 43:900–914

Liu Y, Xiong Y, Bassham DC (2009) Autophagy is required for tolerance of drought and salt stress in plants. Autophagy 5:954–963

Liu B, Cheng Y, Liu Q, Bao JK, Yang JM (2010) Autophagic pathways as new targets for cancer drug development. Acta Pharmacol Sin 31:1154–1164

Lu W, Deng M, Fu G, Wang M, Zeng Z, Han N, Yang Y, Zhu M, Bian H (2016) Suppression of *OsVPE3* enhances salt tolerance by attenuating vacuole rupture during programmed cell death and affects stomata development in rice. Rice 9:65. https://doi.org/10.1186/s12284-016-0138-x

Mansour MMF (2014) Plasma membrane transport systems and adaptation to salinity. J Plant Physiol 171:1787–1800

Mansour MMF, Salama KHA (2004) Cellular basis of salinity tolerance in plants. Environ Exp Bot 52:113–122

Mansour MMF, Salama KHA, Allam HYH (2015) Role of the plasma membrane in saline conditions: lipids and proteins. Bot Rev 81:416–451

Michaeli S, Galili G, Genschik P, Fernie AR, Avin-Wittenberg T (2016) Autophagy in plants—what's new on the menu? Trends Plant Sci 21:134–144

Mimura T, Kura-Hotta M, Tsujimura T, Ohnishi M, Miura M, Okazaki Y, Mimura M, Maeshima M, Washitani-Nemoto S (2003) Rapid increase of vacuolar volume in response to salt stress. Planta 216:397–402

Minina EA et al (2014) Autophagy as initiator or executioner of cell death. Trends Plant Sci 19:692–697

Mitsuya S, Takeoka Y, Miyake H (2000) Effects of sodium chloride on foliar ultrastructure of sweet potato (*Ipomoea batatas* Lam.) plantlets grown under light and dark conditions in vitro. J Plant Physiol 157:661–667

Mittler R (2017) ROS are good. Trends Plant Sci 22:11–19

Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annl Rev Plant Biol 61:443–462

Monetti E, Kadono T, Tran D, Azzarello E, Arbelet-Bonnin D, Biligui B, Briand J, Kawano T, Mancuso S, Bouteau F (2014) Deciphering in early events involved in hyperosmotic stress-induced programmed cell death in tobacco BY-2 cells. J Exp Bot 65:1361–1375

Nam JW, Rissland OS, Koppstein D, Abreu-Goodger C, Jan CH, Agarwal V, Yildirim MA, Rodriguez A, Bartel DP (2014) Global analyses of the effect of different cellular contexts on micro RNA targeting. Mol Cell 53:1031–1043

Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, Bao JK (2012) Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif 45:487–498

Palmgren MG, Nissen P (2011) P-type ATPases. Annu Rev Biophys 40:243–266

Pan YJ, Liu L, Lin YC, Zu YG, Li LP, Tang ZH (2016) Ethylene antagonizes salt-induced growth retardation and cell death process via transcriptional controlling of ethylene-, BAG-and senescence-associated genes in Arabidopsis. Front Plant Sci 7:696. https://doi.org/10.3389/fpls.2016.00696

Pasquinelli AE (2012) MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat Rev Genet 13:271–282

Patterson JH, Newbigin E, Tester M, Bacic A, Roessner U (2009) Metabolic responses to salt stress are described for two barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differed in salinity tolerance. J Exp Bot 60:4089–4103

Perez-Prat E, Narashimhan ML, Binzel ML, Botella MA, Chen Z, Valpuesta V, Bressan RA, Hasegawa PM (1992) Induction of a putative $Ca^{2+}$-ATPase mRNA in NaCl adapted cells. Plant Physiol 100:1471–1478

Petrov V, Hille J, Mueller-Roeber B, Gechev TS (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. Front Plant Sci 6:69

Pieuchot L, Lai J, Loh RA, Leong FY, Chiam K, Stajich J, Jedd G (2015) Cellular subcompartments through cytoplasmic streaming. Dev Cell 34:410–420

Poor P, Kovacs J, Szopko D, Tari I (2013) Ethylene signaling in salt stress- and salicylic acid-induced programmed cell death in tomato suspension cells. Protoplasma 250:273–284

Qudeimat E, Faltusz AM, Wheeler G, Lang D, Holtorf H, Brownlee C, Reski R, Frank W (2008) A PIIB-type $Ca^{2+}$-ATPase is essential for stress adaptation in *Physcomitrella patens*. Proc Nat Acad Sci USA 105:19555–19560

Reape TJ, McCabe PF (2008) Apoptotic-like programmed cell death in plants. New Phytol 180:13–26

Reape TJ, McCabe PF (2010) Apoptotic-like regulation of programmed cell death in plants. Apoptosis 15:249–256

Reape TJ, McCabe PF (2013) Commentary: the cellular condensation of dying plant cells: programmed retraction or necrotic collapse? Plant Sci 207:135–139

Reape T, Brogan N, McCabe P (2015) Mitochondrion and chloroplast regulation of plant programmed cell death. In: Gunawardena A, McCabe P (eds) Plant programmed cell death. Springer, New York, pp 33–53

Rezaei A, Amirjani M, Mahdiyeh M (2013) Programmed cell death induced by salt stress in wheat cell suspension. Int J Forest Soil Eros 3:35–39

Rosenow DT, Quisenberry JE, Wendt CW, Clark LE (1983) Drought tolerant sorghum and cotton germplasm. Agric Water Manag 7:207–222

Sage RF (2004) The evolution of $C_4$ photosynthesis. New Phytol 161:341–370

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141:336–340

Saha P, Sade N, Arzani A, Wilhelmi MMR, Coe KM, Li B, Blumwald E (2016) Effects of abiotic stress on physiological plasticity and water use of *Setaria viridis* (L.). Plant Sci 251:128–138

Sevier C, Kaiser C (2008) Ero1 and redox homeostasis in the endoplasmic reticulum. Biochim Biophys Acta 1783:549–556

Sewelam N, Kazan K, Schenk PM (2016) Global plant stress signaling: reactive oxygen species at the cross-road. Front Plant Sci 7:187

Shabala S (2009) Salinity and programmed cell death: unravelling mechanisms for ion specific signaling. J Exp Bot 60:709–712

Shabala S, Demidchik V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA (2006) Extracellular Ca2+ ameliorates NaCl-induced $K^+$ loss from Arabidopsis root and leaf cells by controlling plasma membrane $K^+$-permeable channels. Plant Physiol 141:1653–1665

Shabala S, Cuin TA, Prismall L, Nemchinov LG (2007) Expression of animal *CED-9* anti-apoptotic gene in tobacco modifies plasma membrane ion fluxes in response to salinity and oxidative stress. Planta 227:189–197

Shimmen T, Yokota E (2004) Cytoplasmic streaming in plants. Curr Opin Cell Biol 16:68–72
Sirisha VL, Sinha M, D'Souza JS (2014) Menadione-induced caspase-dependent programmed cell death in the green chlorophyte *Chlamydomonas reinhardtii*. J Phycol 50:587–601
Su Y, Wu H, Pavlosky A, Zou LL, Deng X, Zhang ZX, Jevnikar AM (2016) Regulatory non-coding RNA: new instruments in the orchestration of cell death. Cell Death Dis 7:e2333. https://doi.org/10.1038/cddis.2016.210
Subbarao GV, Johansen C (1994) Strategies and scope for improving salinity tolerance in crop plants. In: Pessarakli M (ed) Handbook of plant crop stress. Marcel Dekker, New York, pp 559–579
Sullivan A, Lu X (2007) ASPP: a new family of oncogenes and tumour suppressor genes. Br J Cancer 96:196–200
Susin SA, Lorenzo HK, Samzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397:441–446
Swidzinski JA, Sweetlove LJ, Leaver CJ (2002) A custom microarray analysis of gene expression during programmed cell death in *Arabidopsis thaliana*. Plant J 30:431–446
Tanou G, Molassiotis A, Diamantidis G (2009) Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. Environ Exp Bot 65:270–281
Tiwari BS, Belenghi B, Levine A (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol 128:1271–1281
Turan S, Tripathy BC (2015) Salt-stress induced modulation of chlorophyll biosynthesis during de-etiolation of rice seedlings. Physiol Plant 153:477–491
Vacca RA, de Pinto MC, Valenti D, Passarella S, Marra E, De Garra L (2004) Production of reactive oxygen species, alteration of cytoplasmic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat-shock induced cell death in tobacco bright yellow 2 cells. Plant Physiol 134:1100–1112
Van Doorn WG (2011) Classes of programmed cell death in plants, compared to those in animals. J Exp Bot 62:4749–4761
Vavilala SL, Gawde KK, Sinha M, D'Souza S (2015) Programmed cell death is induced by hydrogen peroxide but not by excessive ionic stress of sodium chloride in the unicellular green alga *Chlamydomonas reinhardtii*. Eur J Phycol 50:422–438
Vavilala SL, Sinha M, Gawde KK, Hirolikar SM, D'Souza S (2016) KCl induces a caspase-independent programmed cell death in the unicellular green chlorophyte *Chlamydomonas reinhardtii* (Chlorophyceae). Phycologia 55:378–392
Verslues PE, Batelli G, Grillo S, Agius F, Kim YS, Zhu J, Agarwal M, Katiyar-Agarwal S, Zhu JK (2007) Interaction of $SOS_2$ with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and $H_2O_2$ signaling in *Arabidopsis thaliana*. Mol Cell Biol 27:7771–7780
Wang Z, Song J, Zhang Y, Yang B, Chen S (2009) Expression of baculovirus anti-apoptotic p35 gene in tobacco enhances tolerance to abiotic stress. Biotechnol Lett 31:585–589
Wang J, Li X, Liu Y, Zhao X (2010) Salt stress induces programmed cell death in *Thellungiella halophila* suspension-cultured cells. J Plant Physiol 167:1145–1151
Wang Y, Itaya A, Zhong X, Wu Y, Zhang J, Knaap EV, Olmstead R, Qi Y, Ding B (2011) Function and evolution of a microRNA that regulates a $Ca^{2+}$-ATPase and triggers the formation of phased small interfering RNAs in tomato reproductive growth. Plant Cell 23:3185–3203
Wang P, Zhao L, Hou H, Zhang H, Huang Y, Wang Y, Li H, Gao F, Yan S, Li L (2015) Epigenetic changes are associated with programmed cell death induced by heat stress in seedling leaves of *Zea mays*. Plant Cell Physiol 56:965–976
Wimmers LE, Ewing NN, Bennett AB (1992) Higher plant Ca2C-ATPase: primary structure and regulation of mRNA abundance by salt. Proc Nat Acad Sci USA 89:9205–9209
Wu L, Fan J, Belasco JG (2006) MicroRNAs direct rapid deadenylation of mRNA. Proc Nat Acad Sci USA 103:4034–4039

Wu W, Liu P, Li J (2012) Necroptosis: an emerging form of programmed cell death. Crit Rev Oncol Hematol 82:249–258

Wynn TA, Chawla A, Pollard JW (2013) Macrophage biology in development, homeostasis and disease. Nature 496:445–455

Xu P, Rogers SJ, Roossinck MJ (2004) Expression of antiapoptotic genes *bcl-xL* and *ced-9* in tomato enhances tolerance to viral-induced necrosis and abiotic stress. Proc Nat Acad Sci USA 101:15805–15810

Yamane K, Mitsuya S, Taniguchi W, Miyake H (2012) Salt-induced chloroplast protrusion is the process of exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts into cytoplasm in leaves of rice. Plant Cell Environ 35:1663–1671

Yang T, Zhang L, Hao H, Zhang P, Zhu H, Cheng W, Wang Y, Wang X, Wang C (2015) Nuclear-localized AtHSPR links abscisic acid-dependent salt tolerance and antioxidant defense in Arabidopsis. Plant J 84:1274–1294

Yao N, Bartholomew JE, James M, Greenberg JT (2004) The mitochondrion: an organelle commonly involved in programmed cell death in *Arabidopsis thaliana*. Plant J 40:596–610

Zahra J, Nazim H, Cai S, Han Y, Wu D, Zhang B, Haider SI, Zhang G (2014) The influence of salinity on cell ultrastructures and photosynthetic apparatus of barley genotypes differing in salt stress tolerance. Acta Physiol Plant 36:1261–1269

Zanna C, Ghelli A, Porcelli AM, Martinuzzi A, Carelli V, Rugolo M (2005) Caspase-independent death of Leber's hereditary optic neuropathy cybrids is driven by energetic failure and mediated by AIF and Endonuclease G. Apoptosis 10:997–1007

Zhang H, Dong S, Wang M, Wang W, Song W, Dou X, Zheng X, Zhang Z (2010) The role of vacuolar processing enzyme (VPE) from *Nicotiana benthamiana* in the elicitor-triggered hypersensitive response and stomatal closure. J Exp Bot 61:3799–3812

Zhang X, Wang L, Meng H, Wen H, Fan Y, Zhao J (2011) Maize ABP9 enhances tolerance to multiple stresses in transgenic Arabidopsis by modulating ABA signaling and cellular levels of reactive oxygen species. Plant Mol Biol 75:365–378

Zhang L, Li Y, Lu W, Meng F, Wu C, Guo X (2012) Cotton GhMKK5 affects disease resistance, induces HR-like cell death, and reduces the tolerance to salt and drought stress in transgenic *Nicotiana benthamiana*. J Exp Bot 63:3935–3951

Zhang H, Fan X, Wang B, Song L (2016) Calcium ion on membrane fouling reduction and bioflocculation promotion in membrane bioreactor at high salt shock. Bioresour Technol 200:535–540

Zhu X, Caplan J, Mamillapalli P, Czymmek K, Dinesh-Kuma SP (2010) Function of endoplasmic reticulum calcium ATPase in innate immunity-mediated programmed cell death. EMBO J 29:1007–1018

Zimmermann D, Gomez-Barrera JA, Pasule C, Brack-Frick UB, Sieferer E, Nicholson TM, Pfannstiel J, Stintzi A, Schaller A (2016) Cell death control by matrix metalloproteinases. Plant Physiol 171:1456–1469

Zuppini A, Bugno V, Baldan B (2006) Monitoring programmed cell death triggered by mild heat shock in soybean-cultured cells. Funct Plant Biol 33:617–627