Manipulation of programmed cell death pathways enhances osmotic stress tolerance in plants: physiological and molecular insights

Thi My Linh Hoang, Brett Williams and Sagadevan G. Mundree

Queensland University of Technology (QUT), Centre for Tropical Crops and Biocommodities.

PO Box 2434, Brisbane 4001, Queensland Australia

Email: sagadevan.mundree@qut.edu.au

Abstract

Programmed cell death (PCD) refers to the death of a cell that is genetically "programmed. Alongside cell division and cell migration, PCD enables the organism to strictly control cell numbers and tissue size as well as to protect itself from unwanted cells that threaten homeostasis. Programmed cell death, specifically apoptosis, a physiological form of cell death was first reported in the nematode *C. elegans* in 1972. Since this initial discovery, the functional roles of programmed cell death have been intensely scrutinized and observed across kingdoms ranging from animals to plants. Akin to their mammalian counterparts studies have shown that PCD pathways are vital players in the mediation of plant responses to a range of abiotic stresses, including drought. This chapter will provide: i) an overview of PCD and its roles during development and in response to environmental stimuli; ii) comprehensive literature review of PCD with details of execution and regulation of apoptosis - the most understood form of PCD; iii) PCD and factors that induce cell death in plants; iv) physiological basis of manipulation of PCD pathways enhancing tolerance to

abiotic stress in plant; v) molecular studies of manipulation of PCD pathways as a mechanism of drought stress tolerance in plants and vi) implication and future directions.

Key words: Abiotic stress, apoptosis, PCD, salinity, osmotic stress, drought stress, TUNEL assay

Contents

1. Introduction

Programmed cell death (PCD) is a physiological and genetically controlled process that is evolutionarily conserved across kingdoms. PCD allows multicellular organisms to eliminate excessive or damaged cells which arise during development and in response to abiotic and biotic stress (Williams and Dickman, 2008;Fomicheva et al., 2012). Programmed cell death has been studied extensively in animals and the underlying mechanisms in plants are gradually being discovered.

The roles of PCD during the development of animals were thoroughly reviewed in Fuchs & Steller (2011), especially in regulation of structure sculpting and driving morphogenesis, deletion of unwanted or redundant transient functional structures, control of cell numbers and elimination of unwanted and potentially dangerous cells. In plants, PCD is involved in many stages of development from the embryo to reproduction and aging such as embryogenesis, somatic embryogenesis (Giuliani et al., 2002;Suarez et al., 2004;Hill et al., 2013), sex determination in unisexual species (Dellaporta and Calderon-Urrea, 1994;Beers, 1997), seed development (Young and Gallie, 2000) and senescence (Greenberg, 1996;Yen and Yang, 1998;Simeonova et al.,

2000;Yoshida, 2003). PCD also plays an important role in the elicitation of defence mechanisms. For example, the hypersensitive response, which occurs at the site of pathogen attack and involves programmed cell death of infected as well as uninfected adjacent bystander cells, is one of the strategies that plants employ to prevent pathogen invasion (Lam et al., 2001;Lam, 2004).

Although programmed cell death plays important roles during development and in response to environmental stimuli, it may be beneficial or detrimental to the plant depending on the context (Williams and Dickman, 2008). Being sessile, plants are particularly vulnerable to aberrant environmental conditions including saline soils and water deficit. To mitigate osmotic stress, plants implement a range of strategies, however, if these mechanisms are unable to cope with the prolonged stress imposed the plant will implement selective PCD as a last ditch effort to survive; sacrifice a few cells for the greater good of the organism as a whole (Hara-Nishimura et al., 1991;Greenberg, 1996). Paradoxically, studies have shown that inhibition of PCD during stress promotes survival (Dickman et al., 2001b;Awada et al., 2003;Shabala et al., 2007;Wang et al., 2009b;Li et al., 2010;Hoang et al., 2014;Hoang et al., 2015). In the following sections we will provide an overview of programmed cell death and the physiological and molecular basis of the enhancement of tolerance to osmotic stress associated with drought and salinity through the prevention of programmed cell death in plants.

2. Programmed Cell Death – an Overview

2.1 *PCD – a physiological mechanism for normal development*

Regulation of homeostatic balance between cell division and cell death is fundamental for proper development and well-being of all multicellular organisms (Rudin and Thompson, 1997). Genetically regulated mechanisms in multicellular organisms not only determine which cells live but also which cells die (Raff, 1992;Chinnaiyan and Dixit, 1996). To keep balance with the number

of new cells arising from the body's stem cell populations, about 10 billion cells die every day in adulthood. This normal homeostasis is regulated through apoptosis- one form of programmed cell death (Renehan et al., 2001). Apoptosis is extremely important during various developmental processes and normal physiology (Elmore, 2007). The sculpturing of shape during developing limb to form foetal fingers and toes together with the resorption of the tadpole tail during metamorphosis into a frog are two well-known examples of the programmed cell death involvement in normal development (Zuzarte-Luís and Hurlé, 2002). Evidence indicates that abnormal regulation of programmed cell death especially apoptosis is associated with a wide range of diseases. Insufficient apoptosis results in excessive cell accumulation causing autoimmunity or cancer; inappropriate cell death can lead to chronic degenerative diseases, heart failure, cerebral ischemia, Alzheimer disease, infertility and immunodeficiency (Kondo, 1988;Leijon et al., 1994;Edwards, 1998;Danial and Korsmeyer, 2004;Rami et al., 2008;Lukiw and Bazan, 2010;Whelan et al., 2010;King and Cidlowski, 1998).

2.2. *PCD – as a host defence mechanism against biotic and abiotic stresses*

In addition to development, PCD pathways are also used for adaptation to environmental stresses (Vaux et al., 1994;Mittler and Lam, 1996;Vaux and Strasser, 1996). The Hypersensitive response (HR) of plants to pathogen infection is one example. Plants lack of an active immune system which can produce specialized cells, such as T cells in animal systems that can attack, disable and eliminate pathogen, they instead, induce programmed cell death as one of general defence strategies (Lam et al., 2001;Lam, 2004). During interaction between biotrophic pathogens and host plants, programmed cell death in the form of HR helps plants to prevent infection as biotrophy by definition require living cells for growth and colonisation. However, in some instances plants infected by necrotrophic pathogens e.g. *Sclerotinia sclerotiorum*, cell death is

disadvantageous for the plant as necrotrophic pathogens require dead or dying cells for nutrients. The role of PCD in plant-pathogen interaction, therefore, depends upon the context and in some circumstances the host is involved in the process as a passive participant (Williams and Dickman, 2008).

Cell death in response to abiotic stress provides an advantage to plants in some circumstances but not in others. For example programmed cell death during hypoxia-induced aerenchyma formation in root of maize enables the plants to survive and develop in wetlands where there is limited or no oxygen present (Drew et al., 2000). However, in response to most of other abiotic stresses such as drought, salinity, heat, cold, wounding, UV radiation, aluminium, acifluorfen, sufentrazone, menadione and hydrogen peroxide, prevention of cell death brings more benefit to the plants than execution of cell death as evidenced in many studies (Dickman et al., 2001b;Lincoln et al., 2002;Qiao et al., 2002;Li and Dickman, 2004b;Xu et al., 2004;Doukhanina et al., 2006;Shabala et al., 2007;Wang et al., 2009a;Wang et al., 2009b;Kabbage et al., 2010;Li et al., 2010;Hoang et al., 2014;Hoang et al., 2015).

2.3. PCD – a conserved mechanism

Programmed cell death particularly apoptosis, the physiological form of PCD, has been studied for more than 40 years and is known to occur in many species across all kingdoms. For example human Bcl-2 can partially complement *Caenorhabditis elegans* Ced-9 mutants even though the two genes have limited sequence homology. The animal-derived anti-apoptotic genes *Ced-9* and *Bcl-2* confer tolerance to a wide range of biotic and abiotic stresses, upon over expression in plants (Qiao et al., 2002;Chen and Dickman, 2004;Shabala et al., 2007;Wang et al., 2009a;Paul et al., 2011).

Since the first evidence that a genetic program existed for physiological (programmed) cell death came from studying development in the nematode *Caenorhabditis elegans* (Kerr et al., 1972;Horvitz et al., 1982;Ellis and Horvitz, 1986;Vaux et al., 1988) the study of pathways and regulation of programmed cell death has been carried out on several model systems including *C. elegans*, the vinegar fly *Drosophila melanogaster* and the mouse. The conservation of the core apoptotic machinery has been found across vast evolutionary distances from worm to human, however it is somewhat obscure in plants (Williams and Dickman, 2008;Fuchs and Steller, 2011).

As the core apoptotic machinery is conserved across kingdoms, details of a well-studied programmed cell death model would be helpful to establish an understanding of programmed cell death in plants. In the next section we will review the literature of mammalian PCD pathways.

3. Apoptosis – a Genetically Controlled Cell Death

Three types of programmed cell death have been categorized in mammals based on morphological criteria: apoptosis (type I), autophagy (type II) and necrosis (type III) (Kourtis and Tavernarakis, 2009;Kroemer et al., 2009). Other forms of cell death in mammals related to inflammation response during pathogen invasion have also been observed. This includes pyroptosis (or caspase-1-dependent cell death) and necroptosis (or programmed necrosis) (see review by Bergsbaken *et al*. (2009) and Vandenabeele *et al.* (2010)). Amongst the aforementioned types, apoptosis has been the most studied and the best understood form of PCD in mammals. Apoptosis is a genetically controlled and highly orchestrated cell death. Cells undergoing apoptosis have distinct morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, apoptotic body formation and fragmentation, minor modification of cytoplasmic organelles, and the apoptotic bodies were engulfed by resident phagocytes *in vivo* (Gilchrist, 1998;Bredesen, 2000;Collazo et al., 2006;Kroemer et al., 2009).

3.1. *Execution of apoptosis*

The excecution of apoptosis in mammals relies on the activation of caspases (cysteine aspartic acid specific proteases), a family of higly specific cysteine proteases that are ubiquitously expressed, as inactive precusors (zymogens) with little or no protease activities (Fuchs and Steller, 2011). Caspases can be thought of as the central excecutioners of apoptotic pathways because they bring about most of the visible changes that characterise apoptotic cell death. For example hallmarks of apoptosis such as DNA fragmentation and membrane blebbing are associated with caspase-3 activities (Hengartner, 2000;Zimmermann et al., 2001). Genetic evidence also showed that caspases and their activators play central roles in apoptosis (Cecconi et al., 1998;Los et al., 1999;Zheng et al., 1999;Luthi and Martin, 2007).

The mammalian caspase family can be divided into two subfamilies. The first one is involved in inflammation, where caspases act as pro-cytokine activators and include members of caspases-1, -4, -5, -11, -12, -13 and -14. The other subfamily is involved in apoptosis and includes caspase-2, -3, -6, -7, -8, -9 and -10. The apoptotic subfamily can be further categorised into two subgroups: initiator caspases caspase-2, -8, -9 and -10; and executioners or effector caspases caspase-3, -6 and -7 (Zimmermann et al., 2001;Shi, 2002;Boatright and Salvesen, 2003;Fomicheva et al., 2012).

Since unregulated caspase activity would be lethal for a cell, caspases are synthesized as single-chain zymogens and stored in the cytoplasm as relatively inactive precursors (pro-caspases). Pro-caspases must undergo an activation process during apoptosis to become active caspases (Srinivasula et al., 1998;Yang et al., 1998;Chen and Wang, 2002;Boatright and Salvesen, 2003;Shi, 2004).

The activation of caspases during apoptosis has been reported to occur through three signaling pathways defined as the extrinsic, intrinsic and perforin/ granzyme pathways (Elmore,

2007). The extrinsic pathway is associated with a group of trans-membrane proteins, “death receptors”, which act as surface sensors for the presence of specific extracellular death signals from ligands of tumor necrosis factor (TNF) family (Fomicheva et al., 2012). Death receptors transmit apoptotic signals initiated by specific death ligands and can activate the caspase cascade within seconds of ligand binding (Vaux and Korsmeyer, 1999). The extrinsic pathway of caspase activation is initiated by the ligation of the respective ligand (FasL) to the death receptor (Fas) to form microaggregates at the cell surface. This complex allows the adaptor molecule FADD (Fas-associated protein with death domain) to be recruited to it cytosolic tail by a multi-step mechanism. FADD recruits pro-caspase-8 or pro-caspase-10 by protein-protein interaction via homologous death effector domain (DED) to assemble a death-inducing signaling complex (DISC). During DISC assembly pro-caspase-8 or pro-caspase-10 is activated and released to cytoplasm where it cleaves and hence activates downstream caspase, typically caspase-3. The active caspase-3 cleaves several death substrates leading to the well-known apoptotic hallmarks including nuclear fragmentation, DNA fragmentation, membrane blebbing and other morphological and biochemical changes (Chinnaiyan et al., 1995;Algeciras-Schimnich et al., 2002;Boatright and Salvesen, 2003;Yin et al., 2006;Portt et al., 2011;Fomicheva et al., 2012). The extrinsic pathway is responsible for elimination of unwanted cells during development, immune system education and immune-system-mediated tumor removal (immune-surveillance)(Boatright and Salvesen, 2003).

The intrinsic pathway involves the participation of mitochondrion as a central organelle therefore it is also termed as mitochondrial pathway. The mitochondrial pathway is induced by several stimuli such as UV radiation, DNA damage, voltage changes, oxidative stress [hydrogen peroxide (H_2O_2) or nitrogen oxide (NO)] or growth factor withdrawal (starvation), resulting in the dissipation of mitochondrial membrane potential and increased permeability. The permeabilization

of the mitochondrial outer membrane leads to the release of apoptogenic molecules and proteins including cytochrome c, certain caspases, endonuclease G, Smac/Diablo and apoptosis inducing factor (AIF) from the inter-membrane space of mitochondrion to cytoplasm, resulting in both caspase-dependent and caspase independent PCD (Brenner and Mak, 2009;Paul, 2009). The release of cytochrome c into cytosol and the presence of dATP are essential requirements for apoptotic mediated by mitochondria (Liu et al., 1996;Goldstein et al., 2000;Purring-Koch and McLendon, 2000). Upon releasing, cytochrome c binds to Apaf-1 (apoptotic protease activating factor-1, the mammalian homolog of *C. elegans* Ced-4) in the presence of dATP to form an Apaf-1 complex (Zou et al., 1997;Hu et al., 1999) which then binds to pro-caspase-9 to assemble an oligo-protein complex termed "apoptosome" (Cain et al., 2000;Gupta, 2001;Acehan et al., 2002;Gewies, 2003). The apoptosome activates caspase-9 by dimerization (Purring-Koch and McLendon, 2000;Pop et al., 2006). Active caspase-9 activates downstream caspase, typically caspase-3, resulting in apoptosis. The intrinsic pathway is used to eliminate cells in response to chemotherapeutic drugs, ionizing radiation, mitochondrial damage and certain developmental cues (Boatright and Salvesen, 2003).

The perforin/granzyme pathway involves the cytotoxic T cells and secretion of the transmembrane pore-forming molecule perforin with a subsequent release of cytoplasmic granules which contains two most important components: serine protease granzyme A and B (Elmore, 2007). Granzyme B can activate pro-caspase-10 through the cleavage of this protein at aspartate residues, it can also cleave factors like Inhibitor of Caspase Activated DNAse (ICAD) (Sakahira et al., 1998). Granzyme B can also cleave and activate Bid causing a release of cytochrome c thereby activating the intrinsic pathway of cell death (Russell and Ley, 2002).

Although each pathway is capable of functioning independently, cross-talk between pathways is common. For example, three pathways cooperate to enhance apoptosis through a BH3-only protein member of Bcl-2 pro-apoptotic protein, Bid (Li et al., 1998;Barry and Bleackley, 2002); and more importantly, these pathways converge, leading to the activation of the effector caspase-3 (Schimmer, 2004;Williams and Dickman, 2008).

3.2. *Regulation of apoptosis*

Apoptosis can be regulated in a number of ways including regulators of the death receptors (extrinsic pathway), regulators of mitochondrial-driven PCD (intrinsic pathway) and direct regulator of caspases through Inhibitor of Apoptosis (IAP) proteins. The regulation of apoptosis-mediated by death receptors occurs at multiple levels including regulation of expression of ligands and death receptors and regulation of intracellular regulatory molecules (Chen and Wang, 2002). Meanwhile members of B cell lymphoma 2 (Bcl-2) protein family provides a critical role in regulation of mitochondrial-driven PCD pathway. They either can disrupt or maintain the integrity of mitochondrial membranes thereby promote or prevent the release of apoptogenic proteins such as cytochrome c from inter-mitochondrion membrane space which can activate pro-caspase-9 through assembling of apoptosome leading to apoptosis (Zheng et al., 1998;Heiden et al., 1999;Chen and Wang, 2002;Youle and Strasser, 2008;Fuchs and Steller, 2011;Martinou and Youle, 2011). Bcl-2 family members are characterized by the presence of one or more conserved sequence motifs within α helical segments known as Bcl-2 homology (BH) domains designated BH1, BH2, BH3 and BH4. These BH domains are the only areas of sequence conservation between family members and strongly influence whether the family member is pro or anti-apoptotic (Danial, 2007;Williams and Dickman, 2008). Many members of the Bcl-2 family have a conserved C-terminal transmembrane region (TM) that is responsible for their localization on the outer

mitochondrial membrane, endoplasmic reticulum and nuclear envelope to the cytosolic aspect (Strasser et al., 2000;Soriano and Scorrano, 2010). Bcl-2 family members can be divided into two groups: pro-apoptotic and anti-apoptotic depend upon their functions. At least four models of how Bcl-2 family members regulate apoptosis have been proposed [see review by Strasser *et al.* (2000)]. However exact mechanistic details of how Bcl-2 proteins regulate cell death remain unknown (García-Sáez, 2012).

Although pro-caspases have a low protease activity, this activity is significant; and since pro-caspases are widely expressed in living cells, unregulated caspase activation would be lethal. Therefore cells must have an efficient mechanism to prevent unnecessary caspase activation. Inhibitor of apoptosis (IAP) protein is one of an important family of caspase inhibitors (Fuchs and Steller, 2011). The first member of the IAP family was identified by Crook *et al.*(1993) from the baculovirus *Cyndia pomonella*. Since then several IAPs have been characterized (Birnbaum et al., 1994;Clem and Miller, 1994;Hay et al., 1995;Rothe et al., 1995;Roy et al., 1995;Deveraux et al., 1997;Huang et al., 2000).

IAP family members are characterized by the presence of one to three baculoviral IAP repeat (BIR) domains, a region of approximately 70 amino acids. In some IAP members, BIR domains allow them to bind to and inhibit initiator and effector caspases as well as downstream proteases thereby preventing apoptosis (Deveraux and Reed, 1999;Vaux and Silke, 2005). Unlike FLIP [FLICE (other name of caspase-8)-inhibitory protein] or Bcl-2 anti-apoptotic proteins which can only regulate death receptor or mitochondrial-driven PCD pathways respectively, IAPs are unique in that they are capable of inhibiting both extrinsic and intrinsic pathways due to their inhibition of caspase cleavage at the initial phase of the cascade (Straszewski-Chavez et al., 2004).

The activity of IAP family members is regulated by IAP antagonists, a protein family whose members can bind to the BIR domain of IAP and inactivate the anti-apoptotic function. In Drosophila the anti-apoptotic activity of DIAP1 has been reported to be blocked by *reaper*, *hid* and *grim* encoded proteins (Goyal et al., 2000). In mammalian systems the three well-known IAP antagonists are Smac (second mitochondria-derived activator of caspases), Diablo (Direct IAP binding protein with low pI) and HtrA2/Omi identified by Du *et al.*(2000), Verhagen *et al.*(2000) and Suzuki *et al.*(2001) respectively.

4. Programmed Cell Death in Plants

4.1. *Plant PCD during development and with abiotic and biotic stress*

Most of the functions of PCD (apoptosis and autophagy) that were witnessed in other multicellular organisms such as in animals are also observed in plants. For example, the involvement of PCD in tissue remodeling has been reported in leaf shape remodeling of the lance plant (Gunawardena et al., 2004). PCD functions in deletion of temporary functional structures that are no longer required for the plant development such as suspensor and aleurone layer cells (Pennell and Lamb, 1997;Bozhkov et al., 2005). The aleurone is the outer surrounding layer of endosperm, a store of nutrients materials, in mature seeds. The death of aleurone layer cells during seed germination in cereals is an example of the function of PCD in removing a no longer required structure during plant development. Nutrients required for the growth of the embryo during seed germination are initially obtained from the store in the embryo and subsequently from mobilization of the materials stored in the endosperm. The hydrolytic process of materials stored in endosperm required hydrolytic enzymes which are synthesized in aleurone cells (Kuo et al., 1996;Wang et al., 1996b;Fath et al., 2000). However, aleurone layer cells are not required for young plants and are

therefore programmed to die after contributing their hydrolytic enzymes usually a few days after seed germination (Wang et al., 1996b;Bethke et al., 1999;Fath et al., 2000;Fath et al., 2002).

PCD also plays a key role in the specialization of cells including the development of xylem tracheary elements (Fukuda et al., 1998;Groover and Jones, 1999) or cell death in root cap cells which protect the root meristem (Wang et al., 1996a). Additionally, PCD plays a role in the redistribution of nutrients, for example cell death during senescence recycles nutrients from older to younger organs (Greenberg, 1996;Yen and Yang, 1998;Simeonova et al., 2000;Yoshida, 2003). PCD occurs throughout the plant life cycle in many sites of the plants (Pennell and Lamb, 1997).

In terms of defense, as mentioned in previous section, PCD is induced as general defense strategies in plants to compensate for the absence of immune system as well as the inability to move to escape environmental challenges during pathogens invasion. The decision to kill adjacent uninfected cells to create a "barrier of death" separating the pathogen from healthy tissues help plants minimize the detrimental effects of pathogens invasion (Dangl and Jones, 2001;Lam et al., 2001;Lam, 2004). Hypoxic conditions in maize triggered cell death in the cortex of the roots and stem to form aerenchyma which facilitates an efficient transportation of oxygen from aerial organs to waterlogged stem bases and roots is another example of PCD function to enable plants to cope with unfavorable environmental conditions (Pennell and Lamb, 1997;Drew et al., 2000).

The functions of PCD in plants and animals appear to be conserved with some typical morphological features of PCD in animals such as cell shrinkage, DNA cleavage, DNA fragmentation were also observed during plant PCD, the question about the similarity of molecular mechanisms involved in PCD between the two kingdoms remain unanswered (Fomicheva et al., 2012). Plant cells display several unique features compared to their animal counterparts including the presence of chloroplast, vacuoles and totipotency. Additionally, unlike animal cells, plant cells

are held together by rigid cell walls which prevent active phagocytosis; plants also lack “true” caspases. Despite intense searches, caspases, which are the most characteristic proteases and known to have essential functions in the initiation and execution of apoptosis in animal cells, have yet to be found in plants (Vartapetian et al., 2011;Domínguez and Cejudo, 2012). However a number of caspase-like proteases in plants have been identified including metacaspases (Uren et al., 2000), vacuolar processing enzymes (VPE) (Hatsugai et al., 2004) and subtilisin-like proteases (saspases and phytapases) (Chichkova et al., 2004;Coffeen and Wolpert, 2004;Chichkova et al., 2010). Although plant caspase-like proteases have been identified, their target proteins and the way in which they are activated, regulated and participate in plant PCD pathways awaits further investigation.

It has been suggested in the literature that plants do not exhibit “classical” apoptosis (van Doorn, 2011). Van Doorn (2011) therefore proposed a classification of plants PCD in which 2 categories of PCD were described: vacuolar plant cell death and necrotic plant cell death. There are many cases of plant PCD however, not falling within either of the proposed categories. Classification of plant PCD therefore should base on other criteria such as molecular mechanisms and basic components of PCD apparatus rather than morphology alone (Fomicheva et al., 2012). Other authors, Reape *et al.*(2008), described three different modes of programmed cell death in plants including apoptotic-like PCD (AL-PCD), autophagy and necrosis. Reape *et al.* (2008) also proposed an apoptotic-like regulation of PCD in plants in which mitochondrial membrane permeabilisation plays a central role via the forming of permeability transition pore (PTP), which is induced by the changes in phosphate and/or ATP level, build-up of Ca^{2+} and ROS production following cellular stress (Reape and McCabe, 2010).

4.2. *Plant PCD regulators*

Similar to the case of true caspases, attempts to identify plant homologues of mammalian core regulators of apoptosis using informatics tools at the primary sequence level such as BLAST or FASTA have failed. A search for functional similarity based on prediction from structural similarity has been conducted with the assumption that distantly related proteins may have limited overall (undetectable) sequence homology but key features such as helical structure, hydrophodicity, water accessible surfaces, electrostatic potential, fold and catalytic sites may be conserved; and functional predictions can be made independently of the primary sequence (Doukhanina et al., 2006;Kabbage and Dickman, 2008). Using this approach, a family of *Bcl-2* associated gene product (BAG) proteins of *Arabidopsis* was identified by profile-sequence (PFAM) and profile-profile (FFAS) algorithms (Doukhanina et al., 2006). The BAG family has been identified in yeast and animals, and is believed to function through a complex interaction with signaling molecules and molecular chaperones; under stress conditions, the BAG proteins recruit molecular chaperones to target proteins and modulate their functions by altering protein conformation (Sondermann et al., 2001;Takayama and Reed, 2001). The search of the *Arabidopsis thaliana* genome sequence resulted in recognition of seven homologues of the BAG proteins family with limited sequence but high structural similarity to their human counterparts and contained putative Hsp70 biding sites (Doukhanina et al., 2006). Of the seven homologues of BAG family in *Arabidopsis thaliana,* four with domain organization similar to animal BAGs including AtBAG1-4 which are predicted to localize in cytosol, and three (AtBAG5-7) contain a calmodulin-binding motif near the BAG domain. This is a novel feature associated with plant BAG family and possibly reflecting differences between animal and plant PCD (Kabbage & Dickman 2008). AtBAGs have been speculated to bind Hsp70 in a manner similar to their animal counterparts this is at least the case of AtBAG4. AtBAG4 conferred tolerance to a wide range of abiotic stress in

transgenic tobacco. AtBAG6 may have a role in basal resistance by limiting disease development in *Botrytis cinerea.* The functional differences between AtBAG4 and AtBAG6 lead to a hypothesis that the BAG family has developed specialized roles for cell regulation (Kabbage & Dickman 2008). Similarly to their mammalian counterparts, the proposed function of plant BAG proteins is to coordinate signals for cell growth and to induce cell survival or cell death pathways in response to stress (Doukhanina et al., 2006). *Arabidopsis* BAG family members are localized to a variety of subcellular organelles for a range of cellular functions including the important function in PCD pathways and cytoprotection (Williams et al., 2010).

Despite limited understanding of the molecular mechanisms driving programmed cell death in plants, there is no doubt that PCD occurs in plants during development and during the interaction between plants, the environment and pathogen challenge.

4.3. *Plant PCD-induced factors*

PCD has been reported to be triggered in many plant species during abiotic and biotic stress. For example, salinity stress-induced PCD has been reported in barley (Hatsugai et al., 2006), *Arabidopsis* (Huh et al., 2002), rice (Li et al., 2007;Liu et al., 2007;Jiang et al., 2008), tobacco (Doukhanina et al., 2006;Shabala et al., 2007) and tomato (Li et al., 2010); Drought-induced PCD in tobacco (Awada et al., 2003); fungi-induced PCD (Dickman et al., 2001a). Reactive Oxygen Species (ROS) signals that originate from different organelles such as chloroplast and mitochondria can also trigger PCD (Foyer and Noctor, 2005;Rhoads et al., 2006). In plants, ROS can play a dual role acting as both toxic compounds and secondary messengers in signal transduction pathways in a variety of scenarios (Miller et al., 2008;Miller et al., 2010). ROS levels were reported to increase in plants resulting in significant cellular damage during drought and salinity stress (Borsani et al., 2005;Zhu et al., 2007;Xu et al., 2010). Other factors such as UV

radiation, DNA damage, voltage changes, oxidative stress [hydrogen peroxide (H_2O_2) or nitrogen oxide (NO)] or growth factor withdrawal (starvation) can also trigger cell death in plants (Lam, 2004;Roos and Kaina, 2006;Nawkar et al., 2013).

5. Physiological Basis of Anti-apoptotic Genes Enhance Tolerance to Osmotic Stress in Plants

Anti-apoptotic genes have been reported to enhance tolerance to a range of abiotic and biotic stresses including drought and salinity for more than a decade. However, the physiological basis of stress tolerance especially cell membrane integrity, ion homeostasis, photosynthesis efficiency and relative water content in plant expressing anti-apoptotic genes exposed to osmotic stress associated with salinity was reported recently (Hoang et al., 2014;Hoang et al., 2015). The expression of anti-apoptotic gene in plants suppresses programmed cell death induced by stress thereby promoting survival. The decision of whether a given cell should live or die is essential for the well-being of all multi-cellular organisms (Metazoan). Under several stimuli, this decision depends on the result of a battle between anti-apoptotic (pro-survival) and pro-apoptotic proteins or signals (Li and Dickman, 2004a;Williams and Dickman, 2008). The ratio of anti-apoptotic (pro-survival) versus pro-apoptotic (pro-death) proteins also regulates PCD sensitivity (Fulda et al., 2010). The master switch of the cell life/death decision during osmotic stress associated with salinity stress is the “balance of the pro-death and pro-survival signals” of the system. By exogenous expression of an anti-apoptotic (pro-survival) gene, researchers have pushed the plant to make the “life decision” at the onset of a given stress. Expression of pro-survival genes coincided with reduced pro-death signals such as ROS levels which in turn supported the maintenance of cell membrane integrity and ion homeostasis. This maintenance promoted sustained photosynthetic efficiency which in turn provided energy for growth. Well-maintained growth further dilutes the ion concentration in cells which helps maintain ion homeostasis leading

to the increased membrane integrity, relative water content, net photosynthesis and finally growth and yield.

Figure 1 here

5.1. *Suppression of stress-induced cell death in plants*

Hallmark features of apoptotic-like cell death in plants have been observed during drought and salinity stress. Exogenous expression of a range of PCD related genes from different sources have shown evidence of cell death suppression thereby enhancing tolerance to those stresses in plants (Li et al., 2010;Hoang et al., 2014;Hoang et al., 2015). One of the established methods for detecting apoptotic hallmarks is the Terminal deoxynucleotidyl transferase dUTP Nick End Labelling (TUNEL) assay (Figure 2.2). TUNEL assay is a broad use assay for detecting the nick end of DNA resulted from the DNA fragmentation during apoptosis. Nucleic acid in TUNEL positive cells are selectively stained and fluoresces green, indicating the presence of apoptotic-like bodies, whereas all nucleic acid is counter-stained with propidium iodide and fluoresces red.

Figure 2 here

5.2. *Reactive oxygen species, water retention and cell membrane integrity*

Homeostasis of cellular reactive oxygen species (ROS) levels promotes maintenance of cellular membrane integrity. Studies have shown that ROS-induced cell death can result from oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA, RNA damage (Mittler, 2002).The cell membrane is the first site of signal perception as well as the primary defence against abiotic stresses including salinity and it is one of the most vulnerable targets for ROS due to the predominance of lipids (Ghosh et al., 2011). The maintenance of cell membrane integrity and stability under water stress is an important component of tolerance against water deficit (caused by drought and salinity stress) in plants.

ROS levels in plants expressing anti-apoptosis genes from different sources were maintained at significantly lower levels compared to those in wild type plants (Li et al., 2010;Hoang et al., 2015). The low level of ROS causes less damage to membrane of plant under osmotic stress associated with salinity (Hoang et al., 2015). Among the four types of ROS ($O^{\bullet}_2$, $OH^{\bullet}$, $NO^{\bullet}$ and H_2O_2), H_2O_2 is a relative long-life molecule (1 ms) and it can diffuse some distance cross-linking cell wall structural proteins and more importantly H_2O_2 itself can stimulate further ROS accumulation and function as a local trigger of PCD (Levine et al., 1994;Dat et al., 2000). H_2O_2 can originate from photosynthesis, photorespiration, respiration and from many other cellular processes. It is a potent inhibitor of photosynthesis as it can inhibit CO_2 fixation up to 50% (Foyer and Shigeoka, 2011).

5.3. *Ion homeostasis*

Transgenic plants expressing anti-apoptotic gene from different sources accumulate low Na^+, high K^+ and maintain low Na^+/K^+ ratios during salinity stress (Hoang et al., 2014;Hoang et al., 2015). This is probably a result of the maintenance of cell membrane integrity in transgenic plants expressing the anti-apoptotic gene during salinity stress. High Na^+ levels are toxic to cells because Na^+ has similar physicochemical properties to K^+, it can compete with K^+ for major binding sites in key metabolic processes such as enzymatic reactions, ribosome functions and proteins biosynthesis in the cytoplasm leading to disturbance in metabolism (Shabala and Cuin, 2008;Marschner, 2011;Wang et al., 2013). In addition Na^+ can displace Ca^+ from plasma membranes inducing K^+ leaks out of the cytoplasm across the plasma membrane (Cramer et al., 1985). This results in a decrease in cytosolic K^+ concentration and effects the Na^+/K^+ ratio hence leads to a disturbance of metabolism. Under typical physiological condition, the influx of Na^+ into plant cells is through the H^+-ATPase channel which is responsible for general transport of ions and

nutrients through the plasma membrane; plants maintain a low cytosolic Na^+/K^+ ratio as it is necessary for providing favourable conditions for continued physiological and metabolic activity. During salinity stress increased extracellular Na^+ concentrations create a large electrochemical gradient that favours the passive transport of Na^+ into the cell through K^+ transporters result in high cytosolic Na^+ concentration (Blumwald, 2000). To maintain low cytosolic Na^+ concentrations, plant cells need to extrude Na^+ of the cell or compartmentalize Na^+ into vacuoles. The main mechanism for Na^+ extrusion in plant cells is mediated by the plasma membrane H^+-ATPase (Sussman, 1994). As the cell membrane in wild type plants was damaged during salinity stress it could not use this strategy to pump Na^+ out of the cell hence the Na^+ concentration was recorded at high levels in leaf cells of those plants. On the contrary, transgenic plants expressing anti-apoptotic genes can maintain cell membrane integrity and therefore could use the H^+-ATPase to extrude Na^+ thus maintaining a low concentration of Na^+ in cytoplasm. The high maintenance of low cytosolic Na^+ concentrations facilitates a high concentration of K^+ therefore ensuring a low Na^+/K^+ ratio that could offer an optimal cellular environment for enzymes function thus supporting metabolism. The high cytosolic K^+ concentration in plants expressing anti-apoptotic genes enables the plants to inhibit PCD. Cytosolic K^+ have been suggested to be related to the PCD process as it can affect caspases and caspases-like activities in animal and plants, respectively. Low cytosolic K^+ content in animal tissue correlates with high caspase activity; and the activation of K^+ efflux, the main cause of cytosolic K^+ content decrease, in plant cells leads to PCD hydrolase activation (Hughes Jr and Cidlowski, 1999;Shabala, 2009;Demidchik et al., 2010).

5.4. *Chlorophyll content, maximal photochemical efficiency, photosynthetic rate and growth under osmotic and ionic stress associated with salinity*

Photosynthesis is a fundamental physiological process that provides a source of energy for plants to grow and cope with environmental stresses. Under drought and salinity stress, sensitive cultivars usually display chlorophyll damage, less efficiency of PSII and low photosynthesis efficiency meanwhile the tolerant cultivars can maintain these parameters quite well (Dionisio-Sese and Tobita, 2000;Ismail et al., 2007;Cha-Um et al., 2009). The expression of anti-apoptotic genes in transgenic tobacco led to the maintenance of chlorophyll content as well as the maximal photochemical efficiency of PSII (Shabala et al., 2007). Photosynthetic rate, growth and yield components were also maintained higher in rice expressing anti-apoptotic genes during salinity stress (Hoang et al., 2015). Transgenic rice expressing anti-apoptotic genes *AtBAG4*, *Hsp70, OsBAG4, p35* and *SfIAP* maintain growth rate (shoot growth, dry weight, number of tillers) and yield components (number of panicles per plant and number of spikelets per panicle) during salinity stress. This is probably also a result of the maintenance of high cytosolic K^+ in transgenic rice plants expressing *AtBAG4*, *Hsp70, OsBAG4, p35* and *SfIAP* (Hoang, 2014). It is well known that salinity causes two types of stress on plants: i) osmotic stress which affects plant growth immediately and is caused by excess salt outside the roots; and ii) ionic stress which develops over time and is due to a combination of ion accumulation in the shoot and an inability to tolerate the ions that have accumulated (Munns, 2002;Munns et al., 2006;Munns and Tester, 2008). In low salt environments plant cells can take up water and nutrients from the soil solution to support higher osmotic pressures compared to that of soil solution. However, in high salt environments, the osmotic pressure of the soil exceeds that of plant cells (osmotic stress) and reduces the ability of plants cells to take up soil water and minerals (Kader and Lindberg, 2010). In response to osmotic stress, shoot growth rate decreases immediately (Munns and Tester, 2008). High cytosolic K^+ in transgenic plants expressing pro-survival genes helped the plants to adjust osmotic stress

and maintain high growth rates because one of the important cellular roles of K^+ is to contribute to adjustment of osmotic pressure hence maintain cell turgor (Maathuis and Amtmann, 1999). The maintenance of growth rate leads to higher yield components in transgenic rice expressing anti-apoptotic genes in comparison to wild type plants which had very low cytosolic K^+ under salinity stress condition (Hoang, 2014). Another factor that causes reduced growth rates in high salt environments is inadequate photosynthesis due to limited carbon dioxide uptake as a consequence of stomatal closure (Zhu, 2001). Transgenic rice plants expressing anti-apoptotic genes such as *AtBAG4, Hsp70, OsBAG4, p35* and *SfIAP* maintained high net photosynthesis which provided ample energy for their growth and development.

6. Molecular Basis of Anti-apoptotic Genes Enhance Tolerance to Abiotic Stress in Plant

6.1. *Prevention of protein misfolding*

Abiotic stresses usually cause protein dysfunction therefore one of the most important strategies for cells survival under stress are maintaining proteins in their conformations and preventing the aggregation of non-native proteins (Timperio et al., 2008). Members of the highly conserved heat shock protein family are chaperones that play a key role within the promotion of correct protein folding and proteostasis control (Hartl et al., 2011). A definitive feature of the BAG (Bcl2 anthanogen gene) family of proteins is their ability to bind and facilitate the function of HSPs (Doukhanina et al., 2006;Williams et al., 2010). The expression of anti-apoptotic genes such as *Hsp70, AtBAG4* and *OsBAG4* may assist in the folding of proteins and prevention of protein denaturation in high ROS environments, thus maintaining efficiency of cellular processes and mitigating the production of ROS and plant damage under stress condition (Hoang, 2014). Port *et.al.*(2011) proposed a schematic representation of the processes involved in inducing stress-mediated cell death and its inhibition by key anti-apoptotic proteins. In that scheme stress induced

an unknown substrate that mediated activation of: BH3 only BcL-2 proteins, mitochondria (or other ROS producing system such as NADPH oxidase) and sphingomyelinase. This activation led to the action of at least three pro-apoptotic messengers including active Bax, increased ROS and sphingolipid ceramide thereby causing cell death. Heat shock proteins (HSPs) were proposed to function as anti-apoptotic proteins by blocking that unknown substrate thereby preventing the generation of active Bax, increasing ROS and sphingolipid ceramide.

6.2. *Direct sequestering ROS*

Abiotic stresses cause enhanced generation of ROS in plants due to disruption of cellular homeostasis (Sharma et al., 2012). In plants, ROS are versatile molecules playing dual roles as both toxic compounds and signal transduction molecules that mediate responses to environmental stresses, pathogen infection, developmental stimuli and even PCD (Miller *et al*. 2008; Miller *et al*. 2010). The onset of PCD pathways is triggered by increased ROS levels, among other signals, that originate from a variety of organelles including the chloroplast and mitochondria (Foyer & Noctor, 2005; Rhoads *et al*. 2006). During salinity stress, ROS levels have been reported to increase causing significant injury and eventual death (Borsani et al., 2005;Zhu et al., 2007;Chawla et al., 2013). If left unchecked, copious ROS production can denature proteins and damage cell membrane through the lipid peroxidation. Evidence showed that expression of the anti-apoptotic gene *p35* inhibited H_2O_2-induced PCD in insect cells by directly sequestering ROS. The antioxidant function of p35 has been attributed to the presence of metal-binding sites in the proteins that could enhance its antioxidant property and/or its three-dimensional structure contains some amino acids that confer electro-dynamically stable configuration conducive to ROS-trapping. The antioxidant role of p35 was also supported by the chemical radio-protectors formed by six cysteine residues in its sequence which can react with certain ROS in a constant rate (Sah et al., 1999).

6.3. *Selective degradation of cellular proteins*

The ability to confer tolerance to salinity stress of the anti-apoptotic gene *SfIAP* was attributed to its E3 ubiquitin ligase activity (Kabbage et al., 2010). *SfIAP* has been transformed into tobacco and tomato and reported to confer tolerance to salinity, heat, fumonisin B1 and resistance to necrotrophic fungus *Alternaria alternate* (Kabbage et al., 2010;Li et al., 2010). All aspects of a plant's life are controlled by the regulated synthesis of new polypeptides and the precise degradation of pre-existing proteins (proteolysis). Ubiquitin/26S proteasome is arguably the dominant proteolytic system in plants (Smalle and Vierstra, 2004). Proteolysis via Ubiquitin/26S proteasome pathway requires sequential enzyme activities including a ubiquitin activating enzyme (E1) which forms a thioester bond with the C terminus of ubiquitin in a present of ATP and then transfers the activated ubiquitin to a ubiquitin conjugating enzyme (E2), E2 then transfers ubiquitin directly to a ubiquitin-ligating ezyme (E3) which transfer ubiquitin to the targeted substrate (Smalle and Vierstra, 2004;Kabbage et al., 2010). The expression of *SfIAP* in tobacco resulted in accumulation of ubiquitinated proteins that assist the selective degradation of cellular damaged proteins generation during salt stress. In a presence of a proteasome inhibitor, no significant accumulation of ubiquitylated proteins in plant expressing the anti-apoptotic gene *SfIAP* was observed and *SfIAP* showed no protection during salinity stress in those plants (Kabbage et al., 2010)

In summary, expression of anti-apoptotic genes enhances tolerance to environmental stresses in plants through a number of approaches ranging from cellular to the whole plant. These approaches facilitate the plants to maintain normal physiological and cellular process thereby successfully coping with stresses.

7. Implication and Future Directions

In the next fifty years there will be a massive challenge to sustain an ever-increasing global population. Between the years 1980 – 2000 global population boomed from 4.4 billion through to 6.1 billion, however, food production increased by 50%. By 2050 this problem will be exacerbated with world population predicted to reach 9.6 billion. In order to sustain this increased population, global food within the next 50 years will have to match that which occurred in the last 10,000 years combined. This is a challenge because there is very little potential for future expansion of arable lands whilst climate predictions suggest that a larger portion of the globe will be subjected to erratic environmental conditions and abiotic stress (Eckardt, 2009;FAO, 2009; 2012;Cominelli et al., 2013). Two abiotic stress factors that significantly hinder world crop production are soil water deficit and salinization (Munns, 2011). Researchers have shown that manipulation of PCD pathways can be applied to monocots and dicots for enhancing stress tolerance to a range of abiotic and biotic stresses. Currently we are able to produce crops with enhanced drought and salinity tolerance that survive in the glasshouse, however, once applied in the field the tolerance fails due to combined stresses. One approach with prospective application for the generation of the "next frontier of crop plants" with broad-spectrum tolerance is the exogenous expression of genes that suppress innate Programmed Cell Death (PCD) pathways.

Acknowledgements: The authors thanks Australian Government for financial support through Endeavour Postgraduate Awards.

References

Acehan, D., Jiang, X., Morgan, D.G., Heuser, J.E., Wang, X., and Akey, C.W. (2002). Three-Dimensional Structure of the Apoptosome: Implications for Assembly, Procaspase-9 Binding, and Activation. *Molecular Cell* 9**,** 423-432. doi: http://dx.doi.org/10.1016/S1097-2765(02)00442-2.

Algeciras-Schimnich, A., Shen, L., Barnhart, B.C., Murmann, A.E., Burkhardt, J.K., and Peter, M.E. (2002). Molecular Ordering of the Initial Signaling Events of CD95. *Molecular and Cellular Biology* 22**,** 207-220.

Awada, T., Dunigan, D.D., and Dickman, M.B. (2003). Animal anti-apoptotic genes ameliorate the loss of turgor in water-stressed transgenic tobacco. *Canadian Journal of Plant Science* 83**,** 499-506.

Barry, M., and Bleackley, R.C. (2002). "Cytotoxic T lymphocytes: all roads lead to death." *Nature Reviews Immunology* 2**,** 401-409.

Beers, E.P. (1997). Programmed cell death during plant growth and development. *Cell Death and Differentiation* 4**,** 649-661.

Bergsbaken, T., Fink, S.L., and Cookson, B.T. (2009). Pyroptosis: host cell death and inflammation. *Nat Rev Micro* 7**,** 99-109.

Bethke, P., Lonsdale, J., Fath, A., and Jones, R. (1999). Hormonally regulated programmed cell death in barley aleurone cells *Plant Cell* 11**,** 1033-1046.

Birnbaum, M.J., Clem, R.J., and Miller, L.K. (1994). An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. *Journal of Virology* 68**,** 2521-2528.

Blumwald, E. (2000). Sodium transport and salt tolerance in plants. *Current Opinion in Cell Biology* 12**,** 431-434. doi: http://dx.doi.org/10.1016/S0955-0674(00)00112-5.

Boatright, K.M., and Salvesen, G.S. (2003). Mechanisms of caspase activation. *Current Opinion in Cell Biology* 15**,** 725-731. doi: http://dx.doi.org/10.1016/j.ceb.2003.10.009.

Borsani, O., Zhu, J., Verslues, P.E., Sunkar, R., and Zhu, J.-K. (2005). Endogenous sirnas derived from a pair of natural cis-antisense transcripts regulate salt tolerance in arabidopsis. *Cell* 123**,** 1279-1291. doi: http://dx.doi.org/10.1016/j.cell.2005.11.035.

Bozhkov, P., Filonova, L., and Suarez, M. (2005). Programmed cell death in plant embryogenesis. *Current Topics in Developmental Biology* 67**,** 135-179.

Bredesen, D.E. (2000). Apoptosis: Overview and signal transduction pathways. *Journal of Neurotrauma* 17**,** 801-810.

Brenner, D., and Mak, T.W. (2009). Mitochondrial cell death effectors. *Current Opinion in Cell Biology* 21**,** 871-877. doi: http://dx.doi.org/10.1016/j.ceb.2009.09.004.

Cain, K., Bratton, S.B., Langlais, C., Walker, G., Brown, D.G., Sun, X.-M., and Cohen, G.M. (2000). Apaf-1 oligomerizes into biologically active ∼700-kda and inactive ∼1.4-mda apoptosome complexes. *Journal of Biological Chemistry* 275**,** 6067-6070. doi: 10.1074/jbc.275.9.6067.

Cecconi, F., Alvarez-Bolado, G., Meyer, B.I., Roth, K.A., and Gruss, P. (1998). Apaf1 (CED-4 Homolog) Regulates Programmed Cell Death in Mammalian Development. *Cell* 94**,** 727-737. doi: http://dx.doi.org/10.1016/S0092-8674(00)81732-8.

Cha-Um, S., Supaibulwattana, K., and Kirdmanee, C. (2009). Comparative Effects of Salt Stress and Extreme pH Stress Combined on Glycinebetaine Accumulation, Photosynthetic Abilities and Growth Characters of Two Rice Genotypes. *Rice Science* 16**,** 274-282. doi: 10.1016/s1672-6308(08)60091-8.

Chawla, S., Jain, S., and Jain, V. (2013). Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (Oryza sativa L.). *Journal of Plant Biochemistry and Biotechnology* 22**,** 27-34. doi: 10.1007/s13562-012-0107-4.

Chen, M., and Wang, J. (2002). Initiator caspases in apoptosis signaling pathways. *Apoptosis : An International Journal on Programmed Cell Death* 7**,** 313-319. doi: http://dx.doi.org/10.1023/A:1016167228059.

Chen, S., and Dickman, M.B. (2004). Bcl- family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. *Journal of Experimental Botany* 55**,** 2617-2623.

Chichkova, N.V., Kim, S.H., Titova, E.S., Kalkum, M., Morozov, V.S., Rubtsov, Y.P., Kalinina, N.O., Taliansky, M.E., and Vartapetian, A.B. (2004). A plant caspase-like protease activated during the hypersensitive response. *Plant Cell* 16**,** 157-171. doi: 10.1105/tpc.017889.

Chichkova, N.V., Shaw, J., Galiullina, R.A., Drury, G.E., Tuzhikov, A.I., Kim, S.H., Kalkum, M., Hong, T.B., Gorshkova, E.N., Torrance, L., Vartapetian, A.B., and Taliansky, M. (2010). Phytaspase, a relocalisable cell death promoting plant protease with caspase specificity. *EMBO Journal* 29**,** 1149-1161.

Chinnaiyan, A.M., and Dixit, V.M. (1996). The cell-death machine. *Current Biology* 6**,** 555-562. doi: http://dx.doi.org/10.1016/S0960-9822(02)00541-9.

Chinnaiyan, A.M., O'rourke, K., Tewari, M., and Dixit, V.M. (1995). FADD, a novel death domain-containing protein, interacts with the death domain of fas and initiates apoptosis. *Cell* 81**,** 505-512. doi: http://dx.doi.org/10.1016/0092-8674(95)90071-3.

Clem, R.J., and Miller, L.K. (1994). Control of programmed cell death by the baculovirus genes p35 and iap. *Molecular and Cellular Biology* 14**,** 5212 - 5222.

Coffeen, W.C., and Wolpert, T.J. (2004). Purification and characterization of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in *Avena sativa*. *Plant Cell.* 16**,** 857–873.

Collazo, C., Chacón, O., and Borrás, O. (2006). Programmed cell death in plants resembles apoptosis of animals. *Biotecnología Aplicada* 23**,** 1-10.

Cominelli, E., Conti, L., Tonelli, C., and Galbiati, M. (2013). Challenges and perspectives to improve crop drought and salinity tolerance. *New Biotechnology* 30**,** 355-361. doi: http://dx.doi.org/10.1016/j.nbt.2012.11.001.

Cramer, G.R., Läuchli, A., and Polito, V.S. (1985). Displacement of Ca2+ by Na+ from the Plasmalemma of Root Cells

A Primary Response to Salt Stress? *Plant Physiology* 79**,** 207-211.

Crook, N.E., Clem, R.J., and Miller, L.K. (1993). An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *Journal of Virology* 67**,** 2168–2174. .

Dangl, J.L., and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. *Nature* 411**,** 826-833. doi: http://dx.doi.org/10.1038/35081161.

Danial, N.N. (2007). BCL-2 family proteins: Critical checkpoints of apoptotic cell death. *Clinical Cancer Research* 13**,** 7254-7263. doi: 10.1158/1078-0432.ccr-07-1598.

Danial, N.N., and Korsmeyer, S.J. (2004). Cell death: Critical control points. *Cell* 116**,** 205-219. doi: http://dx.doi.org/10.1016/S0092-8674(04)00046-7.

Dat, J., Vandenabeele, S., Vranová, E., Van Montagu, M., Inzé*, D., and Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences CMLS* 57**,** 779-795. doi: 10.1007/s000180050041.

Dellaporta, S.L., and Calderon-Urrea, A. (1994). The sex determination process in maize. *Science* 266**,** 1501-1505. doi: 10.2307/2885174.

Demidchik, V., Cuin, T.A., Svistunenko, D., Smith, S.J., Miller, A.J., Shabala, S., Sokolik, A., and Yurin, V. (2010). Arabidopsis root K+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. *Journal of Cell Science* 123**,** 1468-1479. doi: 10.1242/jcs.064352.

Deveraux, Q.L., and Reed, J.C. (1999). IAP family proteins—suppressors of apoptosis. *Genes & Development* 13**,** 239-252.

Deveraux, Q.L., Takahashi, R., Salvesen, G.S., and Reed, J.C. (1997). X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388**,** 300-304. doi: http://dx.doi.org/10.1038/40901.

Dickman, M.B., Park, Y.K., Oltersdorf, T., Li, W., Clemente, T., and French, R. (2001a). Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proceedings of the National Academy of Sciences of the United States of America* 98**,** 6957-6962. doi: 10.1073/pnas.091108998.

Dickman, M.B., Park, Y.K., Oltersdorf, T., Li, W., Clemente, T., and French, R. (2001b). Abrogation of disease development in plants expressing animal antiapoptotic genes *Proceeding of the National Academy of Science of the Unitied State of America* 98**,** 6957-6962.

Dionisio-Sese, M.L., and Tobita, S. (2000). Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *Journal of Plant Physiology* 157**,** 54-58. doi: http://dx.doi.org/10.1016/S0176-1617(00)80135-2.

Domínguez, F., and Cejudo, F.J. (2012). A comparison between nuclear dismantling during plant and animal programmed cell death. *Plant Science* 197**,** 114-121. doi: 10.1016/j.plantsci.2012.09.009.

Doukhanina, E.V., Chen, S., Van Der Zalm, E., Godzik, A., Reed, J., and Dickman, M.B. (2006). Identification and functional characterization of the BAG protein family in *Arabidopsis thaliana* *Journal of Biological Chemistry* 281**,** 18793-18801.

Drew, M.C., He, C.-J., and Morgan, P.W. (2000). Programmed cell death and aerenchyma formation in roots. *Trends in Plant Science* 5**,** 123-127. doi: http://dx.doi.org/10.1016/S1360-1385(00)01570-3.

Du, C., Fang, M., Li, Y., Li, L., and Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c–dependent caspase activation by eliminating iap inhibition. *Cell* 102**,** 33-42. doi: http://dx.doi.org/10.1016/S0092-8674(00)00008-8.

Eckardt, N.A. (2009). The Future of Science: Food and Water for Life. *Plant Cell* 21**,** 368-372.

Edwards, M.J. (1998). Apoptosis, the heat shock response, hyperthermia, birth defects, disease and cancer. Where are the common links? *Cell Stress Chaperones.* 3**,** 213–220. .

Ellis, H.M., and Horvitz, H.R. (1986). Genetic control of programmed cell death in the nematode C. elegans. *Cell* 44**,** 817-829. doi: http://dx.doi.org/10.1016/0092-8674(86)90004-8.

Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death. *Toxicologic pathology* 35**,** 495-516. doi: 10.1080/01926230701320337.

Fao (2009). "Declaration of the World Summit on Food Security. 16-18 November, Rome. http://www.fao.org/wsfs/world-summit/wsfs-challenges/en/".).

Fao (2012). "World Water Day 2012 Celebration. UN Conference Centre, Bangkok, 22 March 2012. http://www.fao.org/asiapacific/rap/home/meetings/list/detail/en/?meetings_id=637&year=2012
".).

Fath, A., Bethke, P., Beligni, V., and Jones, R. (2002). Active oxygen and cell death in cereal aleurone cells. *Journal of Experimental Botany* 53**,** 1273-1282. doi: 10.1093/jexbot/53.372.1273.

Fath, A., Bethke, P., Lonsdale, J., Meza-Romero, R., and Jones, R. (2000). Programmed cell death in cereal aleurone. *Plant Molecular Biology* 44**,** 255-266. doi: 10.1023/A:1026584207243.

Fomicheva, A.S., Tuzhikov, A.I., Beloshistov, R.E., Trusova, S.V., Galiullina, R.A., Mochalova, L.V., Chichkova, N.V., and Vartapetian, A.B. (2012). Programmed cell death in plants. *Biochemistry (Moscow)* 77**,** 1452-1464. doi: 10.1134/S0006297912130044.

Foyer, C.H., and Noctor, G. (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. *The Plant Cell Online* 17**,** 1866-1875. doi: 10.1105/tpc.105.033589.

Foyer, C.H., and Shigeoka, S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiology* 155**,** 93-100. doi: 10.1104/pp.110.166181.

Fuchs, Y., and Steller, H. (2011). Programmed cell death in animal development and disease. *Cell* 147**,** 742-758. doi: http://dx.doi.org/10.1016/j.cell.2011.10.033.

Fukuda, H., Watanabe, Y., Kuriyama, H., Aoyagi, S., Sugiyama, M., Yamamoto, R., Demura, T., and Minami, A. (1998). Programming of cell death during xylogenesis. *Journal of Plant Research* 111**,** 253-256. doi: 10.1007/BF02512179.

Fulda, S., Gorman, A.M., Hori, O., and Samali, A. (2010). Cellular Stress Responses: Cell Survival and Cell Death. *International Journal of Cell Biology* 2010**,** 23.

García-Sáez, A.J. (2012). The secrets of the Bcl-2 family. *Cell Death Differ* 19 1733-1740.

Gewies, A. (2003). Introduction to Apoptosis. *ApoReview*.

Gilchrist, D.G. (1998). Programmed cell death in plant disease: The purpose and promise of cellular suicide. *Annual Review of Phytopathology* 36**,** 393-414.

Giuliani, C., Consonni, G., Gavazzi, G., Colombo, M., and Dolfini, S. (2002). Programmed Cell Death during Embryogenesis in Maize. *Annals of Botany* 90**,** 287-292. doi: 10.1093/aob/mcf173.

Goldstein, J.C., Waterhouse, N.J., Juin, P., Evan, G.I., and Green, D.R. (2000). The coordinate release of cytochrome c during apoptosis is rapid, complete and kinetically invariant. *Nature Cell Biology* 2**,** 156-162.

Goyal, L., Mccall, K., Agapite, J., Hartwieg, E., and Steller, H. (2000). Induction of apoptosis by Drosophila reaper, hid and grim through inhibition of IAP function. *EMBO J.* 19**,** 589–597.

Greenberg, J.T. (1996). Programmed cell death: A way of life for plants. *Proceedings of the National Academy of Sciences of the United States of America* 93**,** 12094-12097.

Groover, A., and Jones, A.M. (1999). Tracheary Element Differentiation Uses a Novel Mechanism Coordinating Programmed Cell Death and Secondary Cell Wall Synthesis. *Plant Physiology* 119**,** 375-384. doi: 10.2307/4278635.

Gunawardena, A.H.L.a.N., Greenwood, J.S., and Dengler, N.G. (2004). Programmed Cell Death Remodels Lace Plant Leaf Shape during Development. *Plant Cell* 16**,** 60-73.

Gupta, S. (2001). Molecular steps of death receptor and mitochondrial pathways of apoptosis. *Life Sciences* 69**,** 2957-2964. doi: http://dx.doi.org/10.1016/S0024-3205(01)01404-7.

Hara-Nishimura, I., Inoue, K., and Nishimura, M. (1991). A unique vacuolar processing enzyme responsible for conversion of several proprotein precursors into the mature forms. *FEBS Letters* 294**,** 89-93. doi: http://dx.doi.org/10.1016/0014-5793(91)81349-D.

Hartl, F.U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature* 475**,** 324-332.

Hatsugai, N., Kuroyanagi, M., Nishimura, M., and Hara-Nishimura, I. (2006). A cellular suicide strategy of plants: vacuole-mediated cell death. *Apoptosis : An International Journal on Programmed Cell Death* 11**,** 905-911. doi: http://dx.doi.org/10.1007/s10495-006-6601-1.

Hatsugai, N., Kuroyanagi, M., Yamada, K., Meshi, T., and Et Al. (2004). A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* 305**,** 855-858.

Hay, B.A., Wassarman, D.A., and Rubin, G.M. (1995). Drosophila homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* 83**,** 1253-1262. doi: http://dx.doi.org/10.1016/0092-8674(95)90150-7.

Heiden, M.G.V., Chandel, N.S., Schumacker, P.T., and Thompson, C.B. (1999). Bcl-xL Prevents Cell Death following Growth Factor Withdrawal by Facilitating Mitochondrial ATP/ADP Exchange. *Molecular Cell* 3**,** 159-167. doi: http://dx.doi.org/10.1016/S1097-2765(00)80307-X.

Hengartner, M.O. (2000). The biochemistry of apoptosis. *Nature* 407**,** 770-776. doi: http://dx.doi.org/10.1038/35037710.

Hill, R.D., Huang, S., and Stasolla, C. (2013). Hemoglobins, programmed cell death and somatic embryogenesis. *Plant Science* 211**,** 35-41. doi: http://dx.doi.org/10.1016/j.plantsci.2013.06.010.

Hoang, T.M.L. (2014). *Engineering salinity tolerance in rice by manipulation of exogenous expression of cell death regulators.* PhD, Queensland University of Technology.

Hoang, T.M.L., Moghaddam, L., Williams, B., Khanna, H., Dale, J., and Mundree, S.G. (2015). Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. *Frontiers in Plant Science* 6. doi: 10.3389/fpls.2015.00175.

Hoang, T.M.L., Williams, B., Khanna, H., Dale, J., and Mundree, S.G. (2014). Physiological basis of salt stress tolerance in rice expressing the antiapoptotic gene SfIAP. *Functional Plant Biology* 41**,** 1168-1177. doi: http://dx.doi.org/10.1071/FP13308.

Horvitz, H.R., Ellis, H.M., and Sternberg, P.W. (1982). Programmed cell death in nematode development. *Neuroscience Commentaries* 1**,** 56-65.

Hu, Y., Benedict, M.A., Ding, L., and Núñez, G. (1999). Role of cytochrome c and dATP/ATP hydrolysis in Apaf-1 mediated caspase-9 activation and apoptosis. *EMBO J.* 18**,** 3586–3595.

Huang, Q., Deveraux, Q.L., Maeda, S., Salvesen, G.S., Stennicke, H.R., Hammock, B.D., and Reed, J.C. (2000). Evolutionary conservation of apoptosis mechanisms: Lepidopteran and baculoviral inhibitor of apoptosis proteins are inhibitors of mammalian caspase-9. *Proceeding of the National Academy of Science of the Unitied State of America* 97**,** 1427–1432.

Hughes Jr, F.M., and Cidlowski, J.A. (1999). Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo. *Advances in Enzyme Regulation* 39**,** 157-171. doi: http://dx.doi.org/10.1016/S0065-2571(98)00010-7.

Huh, G.-H., Damsz, B., Matsumoto, T.K., Reddy, M.P., Rus, A.M., Ibeas, J.I., Narasimhan, M.L., Bressan, R.A., and Hasegawa, P.M. (2002). Salt causes ion disequilibrium-induced programmed cell death in yeast and plants. *The Plant Journal* 29**,** 649-659. doi: 10.1046/j.0960-7412.2001.01247.x.

Ismail, A., Heuer, S., Thomson, M., and Wissuwa, M. (2007). Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Molecular Biology* 65**,** 547-570. doi: 10.1007/s11103-007-9215-2.

Jiang, A.-L., Cheng, Y., Li, J., and Zhang, W. (2008). A zinc-dependent nuclear endonuclease is responsible for DNA laddering during salt-induced programmed cell death in root tip cells of rice. *Journal of Plant Physiology* 165**,** 1134-1141. doi: 10.1016/j.jplph.2007.12.008.

Kabbage, M., and Dickman, M.B. (2008). The BAGproteins: a ubiquitous family of chaperone regulators. *Cellular and Molecular Life Sciences* 65**,** 1390 - 1402.

Kabbage, M., Li, W., Chen, S., and Dickman, M.B. (2010). The E3 ubiquitin ligase activity of an insect anti-apoptotic gene (SfIAP) is required for plant stress tolerance. *Physiological and Molecular Plant Pathology* 74**,** 351-362. doi: http://dx.doi.org/10.1016/j.pmpp.2010.06.002.

Kader, M.A., and Lindberg, S. (2010). Cytosolic calcium and pH signaling in plants under salinity stress. *Plant Signaling Behavior* 5**,** 233–238.

Kerr, J.F., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* 26**,** 239-257.

King, K.L., and Cidlowski, J.A. (1998). Cell cycle regulation and apoptosis. *Annual Review of Physiology* 60**,** 601-617.

Kondo, S. (1988). Altruistic Cell Suicide in Relation to Radiation Hormesis. *International Journal of Radiation Biology* 53**,** 95-102. doi: doi:10.1080/09553008814550461.

Kourtis, N., and Tavernarakis, N. (2009). Autophagy and cell death in model organisms. *Cell Death and Differentiation* 16**,** 21.

Kroemer, G., Galluzzi, L., Vandenabeele, P., Abrams, J., Alnemri, E.S., Baehrecke, E.H., Blagosklonny, M.V., El-Deiry, W.S., Golstein, P., Green, D.R., Hengartner, M., Knight, R.A., Kumar, S., Lipton, S.A., Malorni, W., Nuñez, G., Peter, M.E., Tschopp, J., Yuan, J., Piacentini, M., Zhivotovsky, B., and Melino, G. (2009). Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death and Differentiation* 16**,** 3-11. doi: http://dx.doi.org/10.1038/cdd.2008.150.

Kuo, A., Cappelluti, S., Cervantes-Cervantes, M., Rodriguez, M., and Bush, D.S. (1996). Okadaic acid, a protein phosphatase inhibitor, blocks calcium changes, gene expression, and cell death induced by gibberellin in wheat aleurone cells. *Plant Cell* 8**,** 259-269.

Lam, E. (2004). Controlled cell death, plant survival and development. *Nat Rev Mol Cell Biol* 5**,** 305-315.

Lam, E., Kato, N., and Lawton, M. (2001). Programmed cell death, mitochondria and the plant hypersensitive response. *Nature* 411**,** 848-853.

Leijon, K., Hammarström, B., and Holmberg, D. (1994). Non-obese diabetic (NOD) mice display enhanced immune responses and prolonged survival of lymphoid cells. *International Immunology* 6**,** 339-345. doi: 10.1093/intimm/6.2.339.

Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. (1994). H2O2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79**,** 583-593. doi: http://dx.doi.org/10.1016/0092-8674(94)90544-4.

Li, H., Zhu, H., Xu, C.-J., and Yuan, J. (1998). Cleavage of BID by Caspase 8 Mediates the Mitochondrial Damage in the Fas Pathway of Apoptosis. *Cell* 94**,** 491-501. doi: http://dx.doi.org/10.1016/S0092-8674(00)81590-1.

Li, J.-Y., Jiang, A.-L., and Zhang, W. (2007). Salt Stress-induced Programmed Cell Death in Rice Root Tip Cells. *Journal of Integrative Plant Biology* 49**,** 481-486. doi: 10.1111/j.1744-7909.2007.00445.x.

Li, W., and Dickman, M. (2004a). Abiotic stress induces apoptotic-like features in tobacco that is inhibited by expression of human Bcl-2. *Biotechnology Letters* 26**,** 87-95. doi: 10.1023/B:BILE.0000012896.76432.ba.

Li, W., and Dickman, M.B. (2004b). Abiotic stress induces apoptotic-like features in tobacco that is inhibited by expression of human Bcl-2. *Biotechnol Lett* 26**,** 87-95.

Li, W., Kabbage, M., and Dickman, M.B. (2010). Transgenic expression of an insect inhibitor of apoptosis gene, SfIAP, confers abiotic and biotic stress tolerance and delays tomato fruit ripening. *Physiological and Molecular Plant Pathology* 74**,** 363-375. doi: 10.1016/j.pmpp.2010.06.001.

Lincoln, J.E., Craig, R., Overduin, B., Smith, K., Bostock, R., and Gilchrist, D.G. (2002). Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc Natl Acad Sci USA* 99**,** 15217–15221.

Liu, S.-H., Fu, B.-Y., Xu, H.-X., Zhu, L.-H., Zhai, H.-Q., and Li, Z.-K. (2007). Cell death in response to osmotic and salt stresses in two rice (Oryza sativa L.) ecotypes. *Plant Science* 172**,** 897-902. doi: 10.1016/j.plantsci.2006.12.017.

Liu, X., Kim, C.N., Yang, J., Jemmerson, R., and Wang, X. (1996). Induction of Apoptotic Program in Cell-Free Extracts: Requirement for dATP and Cytochrome c. *Cell* 86**,** 147-157. doi: http://dx.doi.org/10.1016/S0092-8674(00)80085-9.

Los, M., Wesselborg, S., and Schulze-Osthoff, K. (1999). The Role of Caspases in Development, Immunity, and Apoptotic Signal Transduction: Lessons from Knockout Mice. *Immunity* 10**,** 629-639. doi: http://dx.doi.org/10.1016/S1074-7613(00)80062-X.

Lukiw, W.J., and Bazan, N.G. (2010). Inflammatory, Apoptotic, and Survival Gene Signaling in Alzheimer's Disease. A Review on the Bioactivity of Neuroprotectin D1 and Apoptosis. *Molecular Neurobiology* 42**,** 10-16.

Luthi, A., and Martin, S. (2007). The CASBAH: a searchable database of caspase substrates. *Cell Death and Differentiation*
14**,** 641-650.

Maathuis, F.J.M., and Amtmann, A. (1999). K+Nutrition and Na+Toxicity: The Basis of Cellular K+/Na+Ratios. *Annals of Botany* 84**,** 123-133. doi: http://dx.doi.org/10.1006/anbo.1999.0912.

Marschner, P. (2011). "Marschner's Mineral Nutrition of Higher Plants". Elsevier Science).

Martinou, J.-C., and Youle, R.J. (2011). Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Developmental Cell* 21**,** 92-101.

Miller, G., Shulaev, V., and Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. *Physiologia Plantarum* 133**,** 481-489. doi: 10.1111/j.1399-3054.2008.01090.x.

Miller, G.a.D., Suzuki, N., Ciftci-Yilmaz, S., and Mittler, R.O.N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment* 33**,** 453-467. doi: 10.1111/j.1365-3040.2009.02041.x.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science* 7**,** 405-410.

Mittler, R., and Lam, E. (1996). Sacrifice in the face of foes: Pathogen-induced programmed cell death in plants. *Trends in Microbiology* 4**,** 10-15. doi: http://dx.doi.org/10.1016/0966-842X(96)81499-5.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment* 25**,** 239–250.

Munns, R. (2011). " Plant Adaptations to Salt and Water Stress: Differences and Commonalities," in *Advances in Botanical Research*, ed. T. Ismail. Academic Press), 1-32.

Munns, R., James, R.A., and Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57**,** 1025-1043.

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59**,** 651-681.

Nawkar, G.M., Maibam, P., Park, J.H., Sahi, V.P., Lee, S.Y., and Kang, C.H. (2013). UV-induced cell death in plants. *International Journal of Molecular Sciences* 14**,** 1608-1628. doi: doi:http://dx.doi.org/10.3390/ijms14011608

Paul, J.-Y. (2009). *The manipulation of apoptosis-related genes to generate resistance to fusarium wilt and water stress in banana.* PhD, Queensland University of Technology.

Paul, J.-Y., Becker, D.K., Dickman, M.B., Harding, R.M., Khanna, H.K., and Dale, J.L. (2011). Apoptosis-related genes confer resistance to Fusarium wilt in transgenic 'Lady Finger' bananas. *Plant Biotechnology Journal* 9**,** 1141-1148. doi: 10.1111/j.1467-7652.2011.00639.x.

Pennell, R.I., and Lamb, C. (1997). Programmed Cell Death in Plants. *Plant Cell* 9**,** 1157-1168.

Pop, C., Timmer, J., Sperandio, S., and Salvesen, G.S. (2006). The Apoptosome Activates Caspase-9 by Dimerization. *Molecular Cell* 22**,** 269-275. doi: http://dx.doi.org/10.1016/j.molcel.2006.03.009.

Portt, L., Norman, G., Clapp, C., Greenwood, M., and Greenwood, M.T. (2011). Anti-apoptosis and cell survival: A review. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1813**,** 238-259. doi: http://dx.doi.org/10.1016/j.bbamcr.2010.10.010.

Purring-Koch, C., and Mclendon, G. (2000). Cytochrome c binding to Apaf-1: The effects of dATP and ionic strength. *Proceedings of the National Academy of Sciences* 97**,** 11928-11931. doi: 10.1073/pnas.220416197.

Qiao, J., Mitsuhara, I., Yazaki, Y., Sakano, K., Gotoh, Y., Miura, M., and Ohashi, Y. (2002). Enhanced resistance to salt, cold and wound stresses by overproduction of animal cell death suppressors bcl-xl and ced-9 in tobacco cells - their possible contribution through improved function of organella. *Plant & Cell Physiology* 43**,** 992–1005.

Raff, M.C. (1992). Social Controls on Cell Survival and Cell Death. *Nature* 356**,** 397-400.

Rami, A., Bechmann, I., and Stehle, J.H. (2008). Exploiting endogenous anti-apoptotic proteins for novel therapeutic strategies in cerebral ischemia. *Progress in Neurobiology* 85**,** 273-296. doi: http://dx.doi.org/10.1016/j.pneurobio.2008.04.003.

Reape, T., and Mccabe, P. (2010). Apoptotic-like regulation of programmed cell death in plants. *Apoptosis* 15**,** 249-256. doi: 10.1007/s10495-009-0447-2.

Reape, T.J., Molony, E.M., and Mccabe, P.F. (2008). Programmed cell death in plants: distinguishing between different modes. *Journal of Experimental Botany* 59**,** 435-444. doi: 10.1093/jxb/erm258.

Renehan, A.G., Booth, C., and Potten, C.S. (2001). What is apoptosis, and why is it important? *BMJ : British Medical Journal* 322**,** 1536-1538.

Rhoads, D.M., Umbach, A.L., Subbaiah, C.C., and Siedow, J.N. (2006). Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. *Plant Physiology* 141**,** 357-366. doi: 10.1104/pp.106.079129.

Roos, W.P., and Kaina, B. (2006). DNA damage-induced cell death by apoptosis. *Trends in Molecular Medicine* 12**,** 440-450. doi: http://dx.doi.org/10.1016/j.molmed.2006.07.007.

Rothe, M., Pan, M.-G., Henzel, W.J., Ayres, T.M., and V. Goeddel, D. (1995). The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 83**,** 1243-1252.

Roy, N., Mahadevan, M.S., Mclean, M., Shutter, G., Yaraghi, Z., Farahani, R., Baird, S., Besner-Johnston, A., Lefebvre, C., Kang, X., Salih, M., Aubry, H., Tamai, K., Guan, X., Ioannou, P., Crawford, T.O., De Jong, P.J., Surh, L., Ikeda, J.-E., Korneluk, R.G., and Mackenzie, A. (1995). The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* 80**,** 167-178. doi: http://dx.doi.org/10.1016/0092-8674(95)90461-1.

Rudin, C.M., and Thompson, C.B. (1997). Apoptosis and Disease: Regulation and Clinical Relevance of Programmed Cell Death. *Annual Review of Medicine* 48**,** 267-281. doi: doi:10.1146/annurev.med.48.1.267.

Russell, J.H., and Ley, T.J. (2002). Lymphocyte-mediated cytotoxicity. *Annual Review of Immunology* 20**,** 323-370. doi: http://search.proquest.com/docview/201638982?accountid=13380.

Sah, N.K., Taneja, T.K., Pathak, N., Begum, R., Athar, M., and Hasnain, S.E. (1999). The baculovirus antiapoptotic p35 gene also functions via an oxidant-dependent pathway. *Proc Natl Acad Sci U S A.* 96**,** 4838–4843.

Sakahira, H., Enari, M., and Nagata, S. (1998). Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* 391**,** 96-99. doi: doi:http://dx.doi.org/10.1038/34214.

Schimmer, A.D. (2004). Inhibitor of Apoptosis Proteins: Translating Basic Knowledge into Clinical Practice. *Cancer Research* 64**,** 7183-7190. doi: 10.1158/0008-5472.can-04-1918.

Shabala, S. (2009). Salinity and programmed cell death: unravelling mechanisms for ion specific signalling. *Journal of Experimental Botany* 60**,** 709-712. doi: 10.1093/jxb/erp013.

Shabala, S., and Cuin, T.A. (2008). Potassium transport and plant salt tolerance. *Physiologia Plantarum* 133**,** 651-669. doi: 10.1111/j.1399-3054.2007.01008.x.

Shabala, S., Cuin, T.A., Prismall, L., and Nemchinov, L.G. (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. doi: 10.1007/s00425-007-0606-z.

Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M. (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany* 2012**,** 26. doi: 10.1155/2012/217037.

Shi, Y. (2002). Mechanisms of Caspase Activation and Inhibition during Apoptosis. *Molecular Cell* 9**,** 459-470. doi: http://dx.doi.org/10.1016/S1097-2765(02)00482-3.

Shi, Y. (2004). Caspase Activation: Revisiting the Induced Proximity Model. *Cell* 117**,** 855-858. doi: http://dx.doi.org/10.1016/j.cell.2004.06.007.

Simeonova, E., Sikora, A., Charzyńska, M., and Mostowska, A. (2000). Aspects of programmed cell death during leaf senescence of mono- and dicotyledonous plants. *Protoplasma* 214**,** 93-101. doi: 10.1007/BF02524266.

Smalle, J., and Vierstra, R.D. (2004). The ubiquitin 26s proteasome proteolytic pathway. *Annual Review of Plant Biology* 55**,** 555-590.

Sondermann, H., Scheufler, C., Schneider, C., Hohfeld, J., Hartl, F.U., and Moarefi, I. (2001). Structure of a Bag/Hsc70 complex: Convergent functional evolution of Hsp70 nucleotide exchange factors. *Science* 291**,** 1553-1557.

Soriano, M.E., and Scorrano, L. (2010). "The Interplay between BCL-2 Family Proteins and Mitochondrial Morphology in the Regulation of Apoptosis
" in *BCL-2 Protein Family*, ed. C. Hetz. (New York: Springer New York), 97-114.

Srinivasula, S.M., Ahmad, M., Fernandes-Alnemri, T., and Alnemri, E.S. (1998). Autoactivation of Procaspase-9 by Apaf-1-Mediated Oligomerization. *Molecular Cell* 1**,** 949-957. doi: http://dx.doi.org/10.1016/S1097-2765(00)80095-7.

Strasser, A., O'connor, L., and Dixit, V.M. (2000). Apoptosis signaling. *Annual Review of Biochemistry* 69**,** 217-245.

Straszewski-Chavez, S.L., Abrahams, V.M., Funai, E.F., and Mor, G. (2004). X-linked inhibitor of apoptosis (XIAP) confers human trophoblast cell resistance to Fas-mediated apoptosis. *Molecular Human Reproduction* 10**,** 33-41. doi: 10.1093/molehr/gah001.

Suarez, M.F., Filonova, L.H., Smertenko, A., Savenkov, E.I., Clapham, D.H., Von Arnold, S., Zhivotovsky, B., and Bozhkov, P.V. (2004). Metacaspase-dependent programmed cell death is essential for plant embryogenesis. *Current Biology* 14**,** R339-R340. doi: http://dx.doi.org/10.1016/j.cub.2004.04.019.

Sussman, M. (1994). Molecular analysis of protein in the plant plasma membrane. *Annu Rev Plant Physiol Plant Mol Biol* 45**,** 211-234.

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. (2001). A Serine Protease, HtrA2, Is Released from the Mitochondria and Interacts with XIAP, Inducing Cell Death. *Molecular Cell* 8**,** 613-621. doi: http://dx.doi.org/10.1016/S1097-2765(01)00341-0.

Takayama, S., and Reed, J.C. (2001). Molecular chaperone targeting and regulation by BAG family proteins. *Nature Cell Biology* 3**,** E237-E241.

Timperio, A.M., Egidi, M.G., and Zolla, L. (2008). Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP). *Journal of Proteomics* 71**,** 391-411. doi: http://dx.doi.org/10.1016/j.jprot.2008.07.005.

Uren, A.G., O'rourke, K., Aravind, L., Pisabarro, M.T., Seshagiri, S., Koonin, E.V., and Dixit, V.M. (2000). Identification of Paracaspases and Metacaspases: Two Ancient Families of Caspase-like Proteins, One of which Plays a Key Role in MALT Lymphoma. *Molecular cell* 6**,** 961-967.

Van Doorn, W.G. (2011). Classes of programmed cell death in plants, compared to those in animals. *Journal of Experimental Botany* 62**,** 4749-4761. doi: 10.1093/jxb/err196.

Vandenabeele, P., Galluzzi, L., Vanden Berghe, and T., K., G. (2010). Molecular mechanisms of necroptosis: An ordered cellular explosion. . *Nature Reviews.Molecular Cell Biology* 11 700-714. doi: doi:http://dx.doi.org/10.1038/nrm2970.

Vartapetian, A.B., Tuzhikov, A.I., Chichkova, N.V., Taliansky, M., and Wolpert, T.J. (2011). A plant alternative to animal caspases: subtilisin-like proteases. *Cell Death Differ.* 18**,** 1289–1297.

Vaux, D.L., Cory, S., and Adams, J.M. (1988). Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* 335**,** 440-442.

Vaux, D.L., Haecker, G., and Strasser, A. (1994). An evolutionary perspective on apoptosis. *Cell* 76**,** 777-779. doi: http://dx.doi.org/10.1016/0092-8674(94)90350-6.

Vaux, D.L., and Korsmeyer, S.J. (1999). Cell Death in Development. *Cell* 96**,** 245-254. doi: http://dx.doi.org/10.1016/S0092-8674(00)80564-4.

Vaux, D.L., and Silke, J. (2005). IAPs, RINGs and ubiquitylation. *Nature Reviews. Molecular Cell Biology* 6**,** 287-297. doi: http://dx.doi.org/10.1038/nrm1621.

Vaux, D.L., and Strasser, A. (1996). The molecular biology of apoptosis. *Proc Natl Acad Sci U S A* 93**,** 2239–2244.

Verhagen, A.M., Ekert, P.G., Pakusch, M., Silke, J., Connolly, L.M., Reid, G.E., Moritz, R.L., Simpson, R.J., and Vaux, D.L. (2000). Identification of DIABLO, a Mammalian Protein that Promotes Apoptosis by Binding to and Antagonizing IAP Proteins. *Cell* 102**,** 43-53. doi: http://dx.doi.org/10.1016/S0092-8674(00)00009-X.

Wang, H., Li, J., Bostock, R., and Gilchrist, D. (1996a). Apoptosis: A Functional Paradigm for Programmed Plant Cell Death Induced by a Host-Selective Phytotoxin and Invoked during Development. *Plant Cell* 8**,** 375–391.

Wang, M., Oppedijk, B., Lu, X., Duijn, B., and Schilperoort, R. (1996b). Apoptosis in barley aleurone during germination and its inhibition by abscisic acid. *Plant Molecular Biology* 32**,** 1125-1134. doi: 10.1007/BF00041396.

Wang, M., Zheng, Q., Shen, Q., and Guo, S. (2013). The Critical Role of Potassium in Plant Stress Response. *Int J Mol Sci.* 14**,** 7370-7390.

Wang, W., Pan, J., Zheng, K., Chen, H., Shao, H., Guo, Y., Hongwu, B., Han, N., Wang, J., and Zhu, M. (2009a). Ced-9 inhibits Al-induced programmed cell death and promotes Al tolerance in tobacco. *Biochemical and Biophysical Research Communications* 383**,** 141–145.

Wang, Z., Song, J., Zhang, Y., Yang, B., and Chen, S. (2009b). Expression of baculovirus anti-apoptotic p35 gene in tobacco enhances tolerance to abiotic stress. *Biotechnol Lett* 31**,** 585-589.

Whelan, R.S., Kaplinskiy, V., and Kitsis, R.N. (2010). Cell Death in the Pathogenesis of Heart Disease: Mechanisms and Significance. *Annual Review of Physiology* 72**,** 19-44. doi: doi:10.1146/annurev.physiol.010908.163111.

Williams, B., and Dickman, M. (2008). Plant programmed cell death: can't live with it; can't live without it. *Molecular Plant Pathology* 9**,** 531–544.

Williams, B., Kabbage, M., Britt, R., and Dickman, M.B. (2010). AtBAG7, an Arabidopsis Bcl-2–associated athanogene, resides in the endoplasmic reticulum and is involved in the unfolded protein response. *PNAS* 107**,** 6088-6093.

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

Xu, Z., Zhou, G., and Shimizu, H. (2010). Plant responses to drought and rewatering. *Plant Signaling & Behavior* 5**,** 649-654.

Yang, X., Chang, H.Y., and Baltimore, D. (1998). Autoproteolytic Activation of Pro-Caspases by Oligomerization. *Molecular Cell* 1**,** 319-325. doi: http://dx.doi.org/10.1016/S1097-2765(00)80032-5.

Yen, C.-H., and Yang, C.-H. (1998). Evidence for Programmed Cell Death during Leaf Senescence in Plants. *Plant and Cell Physiology* 39**,** 922-927.

Yin, Q., Park, H.H., Chung, J.Y., Lin, S.-C., Lo, Y.-C., Da Graca, L.S., Jiang, X., and Wu, H. (2006). Caspase-9 Holoenzyme Is a Specific and Optimal Procaspase-3 Processing Machine. *Molecular Cell* 22**,** 259-268. doi: http://dx.doi.org/10.1016/j.molcel.2006.03.030.

Yoshida, S. (2003). Molecular regulation of leaf senescence. *Current Opinion in Plant Biology* 6**,** 79-84. doi: http://dx.doi.org/10.1016/S1369526602000092.
Youle, R.J., and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nature Reviews. Molecular Cell Biology* 9**,** 47-59. doi: http://dx.doi.org/10.1038/nrm2308.
Young, T.E., and Gallie, D.R. (2000). Programmed cell death during endosperm development. *Plant Molecular Biology* 44**,** 283-301. doi: http://dx.doi.org/10.1023/A:1026588408152.
Zheng, L., Trageser, C.L., Willerford, D.M., and Lenardo, M.J. (1998). T Cell Growth Cytokines Cause the Superinduction of Molecules Mediating Antigen-Induced T Lymphocyte Death. *J. Immunol.* 160**,** 763-769.
Zheng, T.S., Hunot, S., Kuida, K., and Flavell, R.A. (1999). Caspase knockouts: matters of life and death. *Cell Death and Differentiation* 6**,** 1043-1053.
Zhu, J.-K. (2001). Plant salt tolerance. *Trends in Plant Science* 6**,** 66-71. doi: http://dx.doi.org/10.1016/S1360-1385(00)01838-0.
Zhu, J., Fu, X., Koo, Y.D., Zhu, J.K., Jenney, F.E.J., Adams, M.W., Zhu, Y., Shi, H., Yun, D.J., Hasegawa, P.M., and Bressan, R.A. (2007). An enhancer mutant of Arabidopsis salt overly sensitive 3 mediates both ion homeostasis and the oxidative stress response. *Mol Cell Biol* 27**,** 5214-5224.
Zimmermann, K.C., Bonzon, C., and Green, D.R. (2001). The machinery of programmed cell death. *Pharmacology & Therapeutics* 92**,** 57-70. doi: http://dx.doi.org/10.1016/S0163-7258(01)00159-0.
Zou, H., Henzel, W.J., Liu, X., Lutschg, A., and Wang, X. (1997). Apaf-1, a Human Protein Homologous to C. elegans CED-4, Participates in Cytochrome c–Dependent Activation of Caspase-3. *Cell* 90**,** 405-413. doi: http://dx.doi.org/10.1016/S0092-8674(00)80501-2.
Zuzarte-Luís, V., and Hurlé, J.M. (2002). Programmed cell death in the developing limb. *Int. J. Dev. Biol.* 46**,** 871 - 876.

Figure legends here

Figure 1: Schematic proposing salinity-induced cell death switch for salinity stress tolerance in plant

Figure 2: Overexpression of anti-apoptotic gene OsBAG4 showed evidence of cell death suppression during osmotic stress associated with salinity stress (100 mM NaCl) in rice (Oryza sativa L.). WT: Wild type; PI: Propidium Iodide; TUNEL: Terminal deoxynucleotidyl Transferase dUTP Nick End Labelling. Images were taken under a Confocal Microscope. Magnifications as indicated.