Plant Programmed cell death

Subjects: Plant Sciences

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Programmed cell death (PCD) is a genetically controlled suicide process present in all living beings with the scope of eliminating cells unnecessary or detrimental for the proper develop-ment of the organism. In plants, PCD plays a pivotal role in many developmental processes such as sex determination, senescence, and aerenchyma formation and is involved in the defense re-sponses against abiotic and biotic stresses.

cell cultures programmed cell death reactive oxygen species reactive nitrogen species

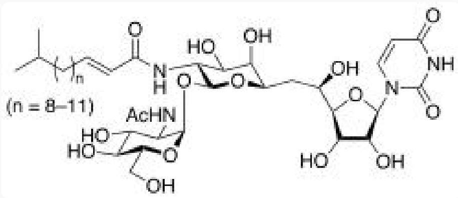
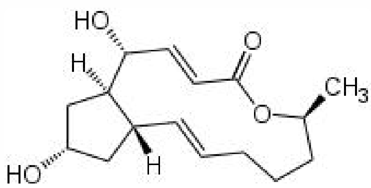
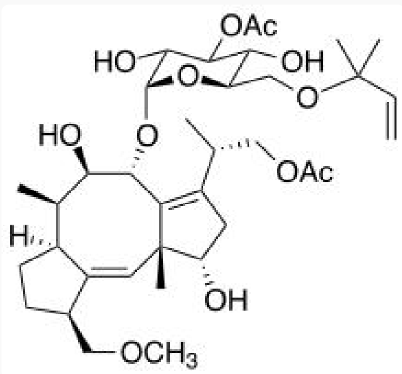
1. Introduction

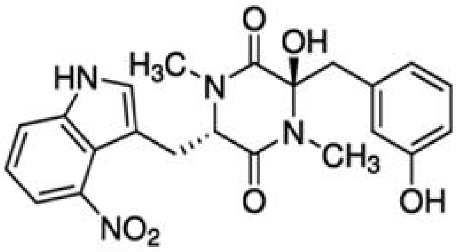
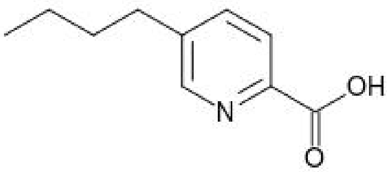
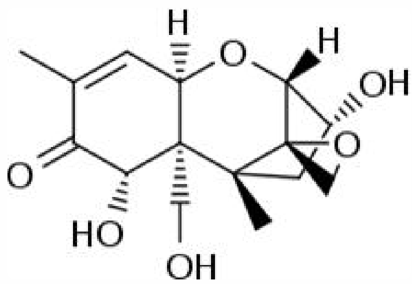
Programmed cell death (PCD) is a genetically controlled suicide process present in all living beings with the scope of eliminating cells unnecessary or detrimental for the proper development of the organism. PCD plays a pivotal role in the plant lifestyle and it is involved in several developmental (senescence, formation of tracheary elements, sex determination, aerenchyma formation, endosperm and aleuron maturation) and pathological contexts (response to stresses and to pathogen attack) [1][2]. Thus, its study is a main goal for plant scientists. PCD process is organized in three phases. The first one is the induction phase, where the cells receive a wide range of extra- or intracellular signals (developmental input, pathogen attack, signals from neighboring cells, abiotic or biotic stresses). The second one is the effector phase, where the signals are elaborated to activate the death machinery. The third one is the degradation phase, where the activity of the death machinery causes the controlled destructuring of fundamental cell components [3]. The degradation phase shows a set of hallmarks that can be used to identify cells undergoing PCD. These hallmarks include shrinkage of cellular and nuclear membrane, activation of specific cysteine proteases called caspases, and activation of specific endonucleases able to cleave DNA in controlled fragments (laddering) [3]. Unlike animals, where well-described forms of PCD (for example apoptosis) are reported, in plants, the PCD process is still poorly understood and the term PCD is widely used to describe cell death observed in different tissues and organs. At present, in plants, at least three forms of PCD have been described and cataloged on the basis of both cellular morphology and the main cellular compartment involved in the process. The “nucleus first form” is observable during the hypersensitive response to pathogen attack and it is similar to animal apoptosis for the presence of specific hallmarks, involvement of mitochondria included. The “chloroplast first form” is observable during foliar senescence, while the “vacuole first form” is observable during the maturation of vascular elements and during aerenchyma formation [4][5][6].

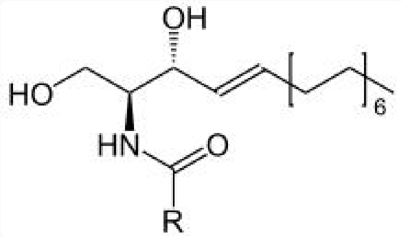
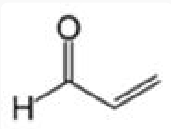
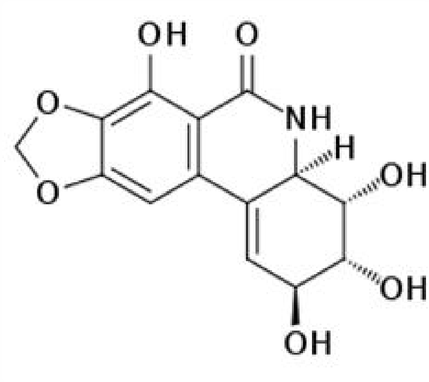
2. PCD Induced in Cell Cultures by Biotic Stress

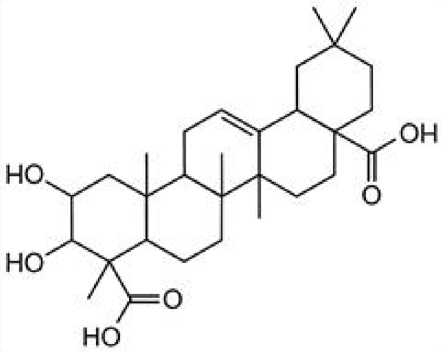
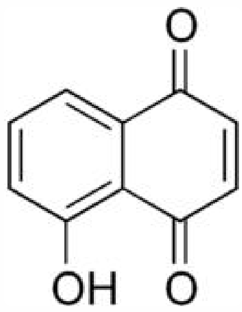
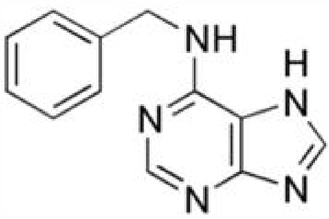
Several toxins and metabolic products obtained by microorganisms and fungi can induce PCD in cell cultures, as summarized in Table 1.

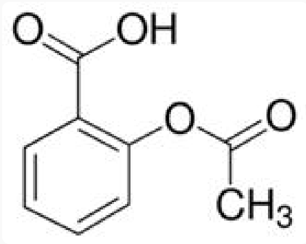
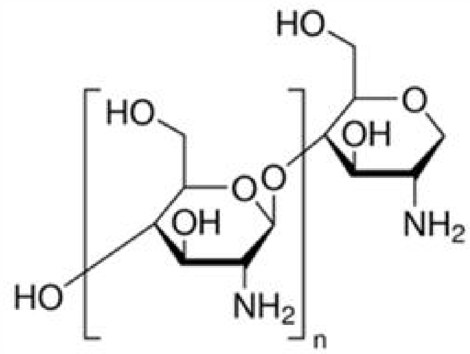
Table 1. Biotic programmed cell death (PCD) inducers in plant cell cultures.

Plant Species	PCD Induced by	Main Characteristics of Induced PCD	Reference
Acer pseudoplatanus L.	 Tunicamycin	H_2O_2 accumulation, changes in cell and nucleus morphology, DNA fragmentation	[7]
Acer pseudoplatanus L.	 Brefeldin A	H_2O_2 accumulation, changes in cell and nucleus morphology, DNA fragmentation	[7]
Acer pseudoplatanus L.	 Fusicoccin	H_2O_2 accumulation, changes in cell and nucleus morphology, DNA laddering	[8]

Arabidopsis thaliana (L.) Heynh.	 Thaxtomin A	Activation of gene transcription and protein synthesis, DNA fragmentation	[9]
Nicotiana tabacum L. cv. Bright Yellow 2	Metabolic products present in the *Alternaria alternata* culture filtrate	Cytoplasm shrinkage, chromatin condensation, DNA laddering	[10]
Nicotiana tabacum L. cv. NC89	 Fusaric acid	H_2O_2 accumulation, lipid peroxidation, caspase-3-like protease activity, mitochondrial dysfunction	[11]
Nicotiana tabacum L. cv. Bright Yellow 2	Culture filtrates of *Erwinia carotovora*	Changes in vacuole shape, endoplasmic actin filaments disassembly	[12]
Nicotiana tabacum L. cv. Bright Yellow 2	 Deoxynivalenol	H_2O_2 accumulation, activation of gene transcription, mitochondrial dysfunction	[13]

Arabidopsis thaliana (L.) Heynh.	 Ceramides	Generation of Ca^{2+} transient, H_2O_2 accumulation	[14]
Solanum lycopersicum L.	Phosphatidic acid	ROS and ethylene accumulation, protoplast shrinkage, nucleus condensation, caspase-3-like protease activity	[15]
Nicotiana tabacum L. cv. Bright Yellow 2	 Acrolein	ROS accumulation, caspase-3-like protease activity	[16]
Nicotiana tabacum L. cv. Bright Yellow 2	 Narciclasin	Cell shrinkage, chromatin condensation, and nuclear DNA degradation	[17]

Populus alba L.	 Medicagenic acid	ROS and RNS accumulation, changes in cell and nucleus morphology, chromatin condensation	[18]
Nicotiana tabacum L. cv. Bright Yellow 2	 Juglone	ROS accumulation, DNA fragmentation and hypomethylation	[19]
Vitis labrusca L.	Alanine	DNA fragmentation, expression of defense-related genes, accumulation of phenolic compounds	[20]
Arabidopsis thaliana (L.) Heynh.	 6-benzylaminopurine	Changes in nucleus morphology, DNA laddering, activation of gene transcription	[21]

Arabidopsis thaliana (L.) Heynh.	 Acetylsalicilic acid	Cell shrinkage, nuclear DNA degradation, mitochondrial dysfunction, induction of caspase-like activity	[22]
Acer pseudoplatanus L.	 Chitosan	ROS and RNS accumulation, nuclear DNA degradation, caspase-3-like protease activity, mitochondrial dysfunction, expression of defense-related genes	[23]

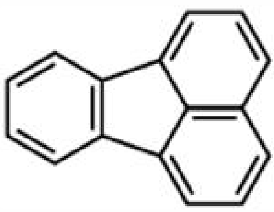
For example, in *Acer pseupdoplatanus* L.-cultured cells, tunicamycin, an inhibitor of N-linked protein glycosylation produced by *Streptomyces lysosuperificus*, and brefeldin A, an inhibitor of protein trafficking from the Golgi apparatus produced by *Eupenicillium brefeldianum*, induce a PCD with apoptotic features such as reactive oxygen species (ROS) accumulation, changes in cell and nucleus morphology, and specific DNA fragmentation. In the same experimental material, fusicoccin a well-known activator of the plasma membrane H^+-ATPase produced by *Phomopsis amygdali* induces PCD with similar characteristics. The well-identified target of these molecules permitted to test the role of specific cell compartments or physiological functions in the induction, development, and execution of plant PCD process. In particular, investigation conducted with fusicoccin showed that the phytotoxin-induced PCD involves changes in actin cytoskeleton [24] and utilizes the plant hormone ethylene as regulative molecule in addition to ROS and reactive nitrogen species (RNS) [25]. Interestingly, inhibition of cytochrome *c* release from the mitochondrion by cyclosporin A markedly prevents the fusicoccin-induced PCD [26], and recently a possible role as signaling molecule for peroxynitrite has been proposed [27]. These results also sustain the fundamental role of cytochrome *c* and peroxynitrite in the induction of PCD process in plants. In *Arabidopsis thaliana* cultures, thaxtomin A, an inhibitor of cellulose biosynthesis produced by *Streptomyces scabiei*, induces a PCD dependent on active gene transcription and de novo protein synthesis and that displays apoptotic-like features such as specific DNA fragmentation. Interestingly, addition of auxin to *Arabidopsis* cell cultures prevents thaxtomin-induced PCD possibly by stabilizing the plasma membrane–cell wall–cytoskeleton continuum [28]. In tobacco BY-2 (*Nicotiana tabacum* L. cv. Bright Yellow 2) cell suspensions metabolic products present in the

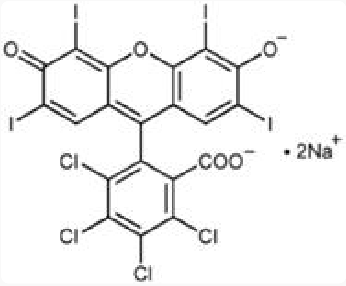
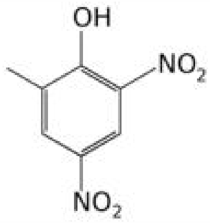
Alternaria alternata culture filtrate induce a PCD dependent on ROS generation that shows cytoplasm shrinkage, chromatin condensation, and DNA laddering. Interestingly, the PCD induced in tobacco BY-2 cells by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* is reduced by culture filtrate of non-pathogenic *Streptomyces* sp. OE7 that through cytosolic Ca^{2+} changes and generation of ROS induces defense responses [29]. This highlights the complexity of the interactions between microorganisms and plants and the need for further investigations. In tobacco cv. NC89-cultured cells, fusaric acid, a non-specific toxin produced mainly by *Fusarium* spp., causes PCD with mechanism that is not well understood that, however, involves ROS overproduction and mitochondrial dysfunction. In fact, pre-treatment of tobacco cells with the antioxidant molecule ascorbic acid and with the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor diphenyl iodonium significantly reduces the fusaric acid-induced accumulation of dead cells as well as the increase in caspase-3-like protease activity. Moreover, oligomycin and cyclosporine A, inhibitors of the mitochondrial ATP synthase and the mitochondrial permeability transition pore, respectively, also reduce the rate of fusaric acid-induced cell death. PCD induced in cell cycle-synchronized tobacco BY-2 cells by application of culture filtrates of *Erwinia carotovora* involves changes in vacuole shape and disassembly of endoplasmic actin filaments. In tobacco BY-2 cultures, deoxynivalenol, a mycotoxin synthesized by *Fusarium culmorum* and *Fusarium graminearum*, induces a PCD sustained by different cross-linked pathways involving ROS generation linked, at least partly, to a mitochondrial dysfunction and to transcriptional downregulation of the alternative oxidase (Aox1) gene and showing regulation of ion channel activities participating in cell shrinkage. Interestingly, this mycotoxin is also able to induce PCD in animal cells, but with different characteristics. This suggests the presence of different ways to induce PCD between animals and plants (original articles cited in [13]). Some metabolites able to induce PCD in plant cultured cells can originate from the degradation of cellular components or are produced by the primary and secondary metabolism of microorganisms and plants. For example, ceramides, lipids derived from the membranes of eukaryotic cells, can induce PCD in *Arabidopsis* cultures in a Ca^{2+}-dependent manner. In fact, the calcium channel-blocker lanthanum chloride substantially reduces the amount of ceramide-induced cell death [14]. Interestingly, in the same material, sphingolipids can reduce apoptotic-like PCD induced by different treatments, ceramides and heat stress included [30]. Moreover, in tomato suspensions, cell death induced by camptothecin, fumonisin B1, and $CdSO_4$ is regulated by phosphatidic acid. This cell death involves ROS and ethylene, depends on caspase-like proteases, and expresses morphological features of apoptotic-like PCD such as protoplast shrinkage and nucleus condensation [15]. Reactive carbonyl species (namely, acrolein, shown in Table 1) derived from lipid peroxidation can activate caspase-3-like proteases to initiate PCD in tobacco BY-2 cultures [16]. In the same experimental material, narciclasine (NCS), a plant growth inhibitor isolated from the secreted mucilage of *Narcissus tazetta* bulbs, can induce typical PCD-associated morphological and biochemical changes, namely, cell shrinkage, chromatin condensation, and nuclear DNA degradation [17]. Among primary and secondary metabolites, the triterpene saponins (namely, medicagenic acid, shown in Table 1) from alfalfa (*Medicago sativa*) applied to *Populus alba* cell cultures induce a PCD dependent on RNS and ROS production and showing changes in nucleus morphology and chromatin condensation [18]. In tobacco BY-2 cultures, juglone (5-hydroxy-1,4-naphthoquinone) causes cell death with ROS overproduction accompanied by formation of apoptic-like nuclear bodies (indication of DNA fragmentation) and DNA hypomethylation [19]. In *Vitis labrusca* suspension cultures, L-alanine is the only amino acid able to induce PCD accompanied by DNA fragmentation, expression of defense-related genes, and accumulation of phenolic compounds [31]. Plant phytoregulators can also activate PCD in plant cell cultures. For example, high levels of cytokinins (namely, 6-benzylaminopurine, shown in Table 1) induce PCD in *Arabidopsis* cultures by accelerating a senescence process characterized by DNA laddering and expression of specific senescence markers. In the same material acetylsalicylic acid, a derivative from the plant hormone salicylic acid induces typical PCD-linked morphological and biochemical changes, namely, cell shrinkage, nuclear DNA degradation, loss of mitochondrial membrane potential, cytochrome *c* release from mitochondria, and induction of caspase-like activity. Finally, in *Acer pseudoplatanus* cultures, chitosan, the non-toxic and inexpensive compound obtained by deacetylation of chitin, the main component of the exoskeleton of arthropods as well as of the cell walls of many fungi, induces a PCD mediated by ROS and RNS accumulation and showing changes in gene expression and specific DNA fragmentation [23].

3. PCD Induced in Cell Cultures by Abiotic Stress

Several abiotic stresses ranking from different chemicals such as heavy metals and dyes to ambient growth conditions can induce PCD in plant cell cultures, as summarized in Table 2.

Table 2. Abiotic PCD inducers in plant cell cultures.

Plant Species	PCD Induced by	Main Characteristics of Induced PCD	Reference
Nicotiana tabacum L. cv. Bright Yellow 2	Cadmium ions	Changes in cell and nucleus morphology, appearance of autophagic bodies	[31]
Nicotiana tabacum L. cv. Bright Yellow 2	Aluminium oxide nanoparticles	Caspase-like protease activity, mitochondrial dysfunction, DNA fragmentation	[32]
Viola tricolor L.	Zinc and lead ions	Changes in cell and nucleus morphology, DNA fragmentation, caspase-like and papain-like cysteine protease activity	[33]
Nicotiana tabacum L. cv. Bright Yellow 2		ROS accumulation, lipid peroxidation, caspase-3-like protease activity, DNA fragmentation	[34]

	Fluoranthene		
Arabidopsis thaliana (L.) Heynh.	 Rose Bengal	ROS accumulation, lipid peroxidation, specific gene activation	[35]
Glycine max (L.) Merr.	 Dinitro-*o*-cresol	Mitochondrial dysfunction, DNA fragmentation, caspase-3-like protease activity	[36]
Populus euphratica Oliv.	1-Butanol	Cell shrinkage, chromatin condensation, nuclear DNA degradation, caspase-3-like protease activity	[37]
Populus euphratica Oliv.	ATP (externally added)	Elevation of cytosolic Ca^{2+} levels, ROS accumulation, mitochondrial dysfunction	[38]
Nicotiana tabacum L. cv. Bright Yellow 2	Heat stress	ROS accumulation, cytoplasmic shrinkage, DNA fragmentation, caspase-3-like protease activity, mitochondrial dysfunction, induction of defense-related genes	[39]
Cakile maritime Scop. *Arabidopsis*	Salt stress	ROS accumulation, lipid peroxidation, specific gene activation, caspase-3-like protease activity, mitochondrial dysfunction	[40]

thaliana (L.) Heynh.			
Arabidopsis thaliana (L.) Heynh.	Ozone	ROS accumulation, Ca^{2+} influx, changes in cell and nucleus morphology	[41]
Vitis vinifera L.	Darkness (in senescent cultures)	ROS accumulation, DNA fragmentation, caspase-3-like protease activity, mitochondrial dysfunction	[42]
Nicotiana tabacum L. cv. Bright Yellow 2	UV-B	ROS accumulation, DNA fragmentation, mitochondrial dysfunction	[43]
Pinus pinaster Ait.	Sugar and phosphate depletion	Changes in cell and nucleus morphology, DNA fragmentation, DNA laddering	[44]
Arabidopsis thaliana (L.) Heynh.	polyethylene glycol, mannose, H_2O_2, ethylene *	Changes in cell and nucleus morphology, DNA fragmentation, specific gene activation. * Cell plasma membrane permeabilization, ROS overproduction, severe oxidative stress	[45]

* Main characteristics of PCD induced by ethylene.

For example, cadmium is a potent inducer of PCD in plants and in tobacco BY-2-cultured cells; this process involves alterations in cell and nucleus morphology and appearance of autophagic bodies . In the same experimental material, aluminum oxide nanoparticles induce a PCD form closely connected to loss of mitochondrial potential, enhancement of caspase-like activity, and DNA fragmentation [32]. In *Viola tricolor* L.-cultured cells, zinc and lead ions stimulate a PCD form showing DNA fragmentation and activation of caspase-like and papain-like cysteine proteases [33]. Interestingly, the indoleamine melatonin protects tobacco BY-2-cultured cells from lead stress by inhibiting cytochrome *c* release, thereby preventing the activation of the cascade of processes leading to cell death [46]. Other important environmental pollutants able to induce PCD in cultured plant cells are aromatic compounds. In fact, fluoranthene causes DNA fragmentation and oxidative stress in tobacco BY-2 suspension cultures [34]. Rose Bengal dye in *Arabidopsis thaliana* cell suspension cultures requires functional chloroplasts to activate a PCD process showing ROS accumulation and specific gene activation [35], and the herbicide dinitro-*o*-cresol induces DNA fragmentation, activation of caspase-3-like proteins, and release of cytochrome *c* from mitochondria in soybean (*Glycine max*) suspension cell cultures [36]. Other chemicals able to induce PCD are 1-butanol, which in *Populus euphratica* cell cultures causes shrinkage of the cytoplasm, DNA fragmentation, condensed or stretched chromatin, and the activation of caspase-3-like proteases [37] and ATP, which when externally added to the same cell cultures causes elevation of cytosolic Ca^{2+} levels, ROS accumulation, and cytochrome *c* release [38]. As far as environmental conditions are concerned, heat stress (HS) is a potent inducer of PCD in plants, where it causes important yield losses. HS study in cultured cells has permitted to elucidate some aspects of its induction, thus helping in the reduction of losses. For example, in tobacco BY-2-cultured cells, HS induces PCD, showing apoptotic features such as cytoplasmic shrinkage, DNA fragmentation, ROS accumulation, activation of caspase-3-like proteases, and induction of defense-related genes [39][47]. Some of these effects of HS are prevented by selenium [47] and depend on peroxynitrite accumulation [48], thus sustaining the fundamental role of oxidative stress in the induction of HS-dependent PCD. This view is also sustained by the analysis of the soluble proteome of tobacco cells subjected to HS and by custom microarray analysis of gene expression during PCD of *Arabidopsis thaliana*-cultured cells. Both these molecular investigations show the induction of genes related to oxidative stress resistance [49][50]. Another environmental condition that is able to induce PCD is salinity. Interestingly, the comparison of the responses to salt stress of suspension-cultured cells from the halophyte *Cakile maritima* and the glycophyte *Arabidopsis thaliana* shows that both species present similar dysfunction of mitochondria and caspase-3-like activation but the salt-tolerant *C. maritima* can better resist to stress due to a higher ascorbate pool able to mitigate the oxidative stress generated in response to NaCl [40]. O_3 exposure also induces PCD dependent on ROS generation in cell suspensions of *Arabidopsis thaliana* [41]. Light also seems to be an important environmental factor able to regulate PCD. Darkness enhances cell death but flavonoids and darkness lower PCD during senescence of *Vitis vinifera* cell suspensions [42], pointing out the complexity of PCD regulation in plants. In tobacco BY 2-cultured cells, UV-B overexposure induces a PCD form showing typical apoptotic morphological features such as cell shrinkage, condensation of chromatin in perinuclear areas, and formation of micronuclei [43]. The nutritional aspect is also important. In fact, simultaneous depletion of sugar and phosphate is associated with PCD, showing nuclear DNA degradation in suspension cultures of maritime pine (*Pinus pinaster* Ait.) [44].

Very interesting results have been obtained from experiments performed in a cell cycle-synchronized *Arabidopsis thaliana* cell suspension culture treated with four physiological stressors (polyethylene glycol, mannose, H_2O_2, ethylene) in the late G2 phase. In these cultures, depending on the cell death inducer, there are significant differences in the appearance of specific PCD hallmarks. In fact, polyethylene glycol, mannose, and H_2O_2 cause DNA fragmentation and cell permeability to vital stains, and produce corpse morphology corresponding to apoptotic-like PCD. Instead, ethylene (a plant hormone associated with senescence) causes permeability of cells to vital stains without concomitant nuclear DNA fragmentation and cytoplasmic retraction but with very high ROS production, leading to severe oxidative stress [45]. Similarly, in tobacco BY 2-cultured cells, zinc oxide nanoparticles cause cell death depending on oxidative stress and lipid peroxidation [51], and in grapevine suspension cell cultures, different concentrations of silver ions cause cell death with different characteristics [52]. Thus, depending on the genotype/species and level of stress, the same factors may cause different responses. Low stress levels permit the repair of cell damage, moderate stress levels may induce PCD, and uncontrollable stress levels potentially lead to accidental cell death (necrosis, see also Section 5). This is particularly evident with abiotic stressors such as heavy metals and externally added compounds such as plant hormones and H_2O_2 (original articles cited in [53]).

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