

Programmed Cell Death and Postharvest Deterioration of Horticultural Produce

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Abstract

Programmed cell death (PCD) is a process where cells or tissues are broken down in an orderly and predictable manner, whereby nutrients are re-used by other cells, tissues or plant parts. The process of (petal) senescence shows many similarities to autophagic PCD in animal cells including a massive breakdown of protein, DNA and RNA, the formation of autophagic vacuoles for the breakdown of cytoplasm and organelles therein and, the eventual rupture of these vacuoles that kills the cell. Common storage disorders such as scald, internal browning, core breakdown and senescent breakdown in fruit and formation of a variety of storage-related problems in vegetables that are induced by severe conditions such as low temperature, low oxygen and increased carbon dioxide concentrations, are accompanied by death and sometimes disappearance of cells. This type of cell death shows similarities to autophagic cell death during aerenchyma formation in flooded roots. This paper discusses different types of cell death in relation to flower petal senescence and storage disorders in fruit and vegetables.

TYPES OF PCD

According to the general definition, programmed cell death (PCD) applies to cell death that is part of the normal life of multi cellular organisms. If so defined, PCD is found throughout the animal and plant kingdoms. It is an active process in which a cell suicide pathway is activated resulting in controlled disassembly of the cell. Besides cell death as a result of normal development (developmental PCD), cell death resulting from environmental stress (e.g., infection by pathogenic organisms, wounding and low concentrations of toxins, severe environmental conditions) also often occurs through controlled disassembly of the cell and can therefore also be termed PCD.

In animal science a type of cell death showing large scale autophagy but no uptake of the cell contents by other cells, was termed Autophagic PCD (Type II PCD). It shows features such as the disappearance of cytoplasm and organelles, formation of double membrane autophagosomes containing cytoplasm and organelles, and the formation of autolysosomes.

Apart from Autophagic PCD, other types of PCD have been identified in animal cells (Table 1). Most prominent is apoptosis (Type I PCD). Other types of programmed cell death have also been assigned based on cell death morphology but these have not been studied intensely and are presumably less important (Table 1).

Apoptosis is the main type of PCD in animal cells. For a considerable period of time the terms PCD and apoptosis have been used as synonyms by scientists working with animal cells. Apoptosis can easily be distinguished from autophagy, as the apoptotic cell is finally degraded inside another cell, whereas the autophagic cell is degraded by itself (self eating). Other features that have often been associated with apoptosis are cell shrinkage, blebbing of the plasma membrane, condensation and fragmentation of the nucleus and internucleosomal cleavage of DNA. The final stage of apoptosis is the fragmentation of the cell into cellular debris-containing vesicles called “apoptotic bodies”

that are being phagocytosed by other cells (Hengartner, 2000). At the biochemical level, the apoptosis signal is transduced by a specific class of cysteine proteases called caspases, and by transport of proteins out of mitochondria (Hail et al., 2006).

Thus, an important difference between autophagy and apoptosis is that during autophagy the cell content is degraded by the cell itself, i.e., by the lysosome of the same cell, whereas during apoptosis the cell often shows protuberances or the cell is split into apoptotic bodies. These protuberances or apoptotic bodies are taken up (phagocytosed) by other cells, and their content is degraded in the lysosome of the living “eater” cells.

Striking and, initially confusing, is that both type I and type II cell death may be accompanied by chromatin condensation, DNA degradation, and nuclear fragmentation. Such features were first observed in apoptotic cells and have therefore become presumed to be “apoptotic features”, and they sometimes are still erroneously called as such. In addition, it has been found that caspases, first thought to be specific for apoptosis, may also be necessary for autophagic PCD in certain systems (Baehrecke, 2003; Lockshin and Zakeri, 2004). It has now been recognized that the same cell may use different ways to die, in particular by using other cells for final degradation or no such cells, depending on the triggers and the cellular context (Baehrecke, 2002; Lockshin and Zakeri, 2004).

A third type of cell death that shows neither degradation of cell material in the lysosome of the same cell nor in the lysosome of another cell is often called Type III PCD, non-lysosomal PCD or apoptotic-like PCD. Type III PCD is also accompanied by some of the “apoptotic” features.

PCD IN SENESCING FLOWER PETALS

Rapid senescence of flower petals is highly undesirable from a postharvest perspective. However, from an ecological point of view senescence should be regarded a functional process. In many flowers, the petals rapidly senesce following pollination, presumably to direct pollinators to other flowers on the plant, to prevent wasting energy necessary to maintain the petals any longer than necessary, and to retrieve nutrients from the senescing tissue. For many decades petal senescence has been a fruitful model to study the mechanism of cell death.

Major early work, using morning glory (*Ipomoea tricolor*) as a model, has identified massive breakdown of proteins and nucleic acids and showed that petal senescence is accompanied by autophagic processes. In senescing petal cells, numerous vesicles and structures reminiscent of mitochondria were found in the vacuole and this compartment was recognized as the main site of autophagic activity. The breakdown of the tonoplast was regarded as the last phase of the senescence process. These observations indicated that senescence proceeds in an ordered and predictable way culminating in tonoplast rupture and death of the cells (Winkenbach, 1970a, b; Matile and Winkenbach, 1971; Wiemken-Gehrig et al., 1974; Wiemken et al., 1976).

Others found similar evidence in other species. In senescing carnation (Smith et al., 1992), *Hemerocallis* (Stead and van Doorn, 1994) and *Iris* (van Doorn et al., 2003) petals, for example, an increase in vacuolar size was observed that was accompanied by a decrease in the amount of cytoplasm and with the disappearance of most organelles, including most of the endoplasmic reticulum and attached ribosomes.

In addition to these autophagic features, a vast amount of evidence for the occurrence of what has often been called “apoptotic-like” features in senescing petals is currently available. DNA degradation is a general feature of senescing petals (e.g., Xu and Hanson, 2000; van der Kop et al., 2003; Jones et al., 2005). DNA in animal cells is often first degraded into large parts (about 50 kbp), then into smaller parts consisting of about 180 bp or multiples of 180 bp. In senescing petals DNA degradation into fragments containing multiples of about 180 bp, showing a ladder pattern on gel, has so far only been observed in a limited number of species e.g., in pea, petunia, *Freesia* and *Alstroemeria* petals (Orzaez and Granell, 1997; Xu and Hanson, 2000; Yamada et al., 2001; Wagstaff et al., 2003). It is not clear if the absence of visible laddering on gels represents lack of laddering. Judged from the DNA gels presented by several authors in

plant senescence/PCD it seems that only a small portion (<5%?) of the DNA actually shows laddering. If no laddering is reported, it may therefore have escaped observation.

Using methods to visualize nuclear features (electron microscopy, TdT mediated dUTP-biotin nick end labelling [TUNEL], DNA staining and flow cytometry) several nuclear ultrastructural changes were observed in senescing petal cells. The nuclei may show blebbing and chromatin often clumps into patches that are found throughout the nucleus or at the nuclear periphery (e.g., Serafini-Facassini et al., 2002; Wagstaff et al., 2003) or, nuclei may show a decrease in size due to chromatin condensation, fragment into separate masses and may contain degraded DNA (e.g., Hoeberichts et al., 2005; Yamada et al., 2006a, b, 2003).

Generally, nuclear morphological changes and in situ DNA degradation are observed in early stages of senescence. In *Gypsophila*, for example, TUNEL-positive nuclei were observed in cells throughout the petal well before the rise in ethylene production, at a time the flower had fully opened but did not yet show a visible sign of senescence (Hoeberichts et al., 2005). This indicates that, at least in *Gypsophila*, the majority of petal cells show nuclear morphological changes and DNA breakdown very early in the senescence process. A similar result was obtained in *Iris* and *Alstroemeria* flowers. However, in these species a clear distinction with respect to the timing of cell death was observed between the mesophyll and the epidermis cells. Most of the mesophyll cells in *Iris* petals were already dead and gone by the time the petal showed visible signs of senescence (van Doorn et al., 2003). In *Alstroemeria* some degenerative changes were found in the nuclei of petal cells even before the flower had fully opened (Wagstaff et al., 2003). Together these observations support the idea that cell death in petal senescence can begin very early, can be regulated by ethylene, and is an integral part of the developmental program of the petal.

PCD IN CULTURED PLANT CELLS

Cultured plant cells and protoplasts have been widely used as model systems to study PCD. Synchronized cell death can reproducibly be induced in such cultures by treatment with mycotoxins, heavy metals and other chemicals or by physical treatments (heat shock, UV light). In the majority of cases, cell death in such systems is accompanied by a characteristic cell morphology (chromatin condensation, cytoplasm shrinkage, nuclear fragmentation), by DNA degradation (sometimes DNA laddering) and sometimes by cytochrome-c release from mitochondria (Reape et al., 2008). Given the similarity to the observed changes in apoptotic animal cells, this type of plant cell death is often called “apoptotic-like PCD”. As the characteristic feature of apoptosis (degradation of cell contents in lysosome of other cells) is lacking in these plant systems, this type of cell death may in fact better be called “Non-lysosomal PCD” (van Doorn and Woltering, 2005).

Functional plant equivalents of animal cell caspases are presumably involved in the process. Among these are the vacuolar processing enzymes (VPEs) and some other cysteine and serine proteases (de Jong et al., 2000; Hara-Nishimura et al., 2005). The production of reactive oxygen species (ROS; for example hydrogen peroxide; de Jong et al., 2002) and reactive nitrogen species (RNS; for example NO) by mitochondria and by the plasma membrane-associated NADPH-oxidase is instrumental in this type of cell death (Fig. 1). Activation of phospholipases-C and D and the production of second messengers phosphatidic acid and calcium are involved in this type of cell death (Yakimova et al., 2006). Although ethylene alone does not induce cell death in these systems, it seems an important stimulator of the process as blocking ethylene production or perception usually greatly diminishes the effect of the chemical and physical cell death inducers.

PCD DUE TO SEVERE ENVIRONMENTAL CONDITIONS

Severe environmental conditions such as the conditions during storage of horticultural produce, in which the temperature and the oxygen concentration are kept low

in order to slow down ageing of the product, may often lead to typical storage disorders such as chilling injury-associated damages and low oxygen damage. These processes may end up in a larger number of visible symptoms (internal browning, superficial scald, necrotic lesions, core breakdown, senescent breakdown) that have in common that cells at specific locations have died and sometimes disappeared (Fig. 2). Although it is clear that oxidative stress and perhaps ethylene are often an integral part of the process, these damages have not been investigated in great detail at the biochemical and morphological level.

Closest to such cell death and disappearance processes due to environmental conditions in horticultural produce is the process of aerenchyma formation in roots during flooding. Due to a lack of sufficient oxygen and the accumulation of ethylene in flooded roots, air channels are formed in the root through the death and disappearance of specific cells (He et al., 1996). Also in roots under mechanical impedance, such as in compacted soils, aerenchyma may be formed. One obvious reason for the formation of such air channels is the restoration of sufficient air flow into the roots from the above ground parts. In both flooded and compacted soils the direct inflow of air from the surrounding soil is hampered. Another reason may be minimizing the amount of respiratory active tissue thereby decreasing the need for oxygen (Drew et al., 2000). The process of aerenchyma formation has been studied intensively and it has been shown that the dying cells show clear signs of autophagy and show the accompanying “apoptotic” features such as the formation of TUNEL-positive nuclei indicating in-nucleus DNA degradation (Drew et al., 2000; Gunawardena et al., 2001). Phospholipase-C and ethylene signalling pathways are instrumental in cell death during aerenchyma formation.

It may therefore be suggested that the death (and sometimes disappearance) of cells in horticultural produce as a result of severe storage conditions may be a form of autophagic PCD. With this concept in mind it is understandable that the appearance of chilling injury may be delayed or prohibited by a periodical increase in temperature as also autophagic processes in senescing plant organs have shown to be reversible once conditions have changed to the good (van Doorn and Woltering, 2004).

The idea that storage disorders are a form of autophagic PCD should be further verified and may direct research activities to treatments that specifically tackle PCD as a way to optimize storage conditions and to increase quality of stored produce.

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Tables

Table 1. Types of PCD recognized in animal cells.

Type	Name	Features
I	Apoptosis	Shrinkage of nucleus and cytoplasm, membrane blebbing, nuclear condensation, DNA degradation and nuclear fragmentation, formation of apoptotic bodies, degradation of cell contents in lysosome of other cells
II	Autophagy	Formation of autophagosomes and autolysosomes, degradation of cell contents in same cell, nuclear condensation, DNA degradation and nuclear fragmentation
III	Non-lysosomal	Shrinkage of cytoplasm, no degradation of cell contents, nuclear morphological changes

Figures

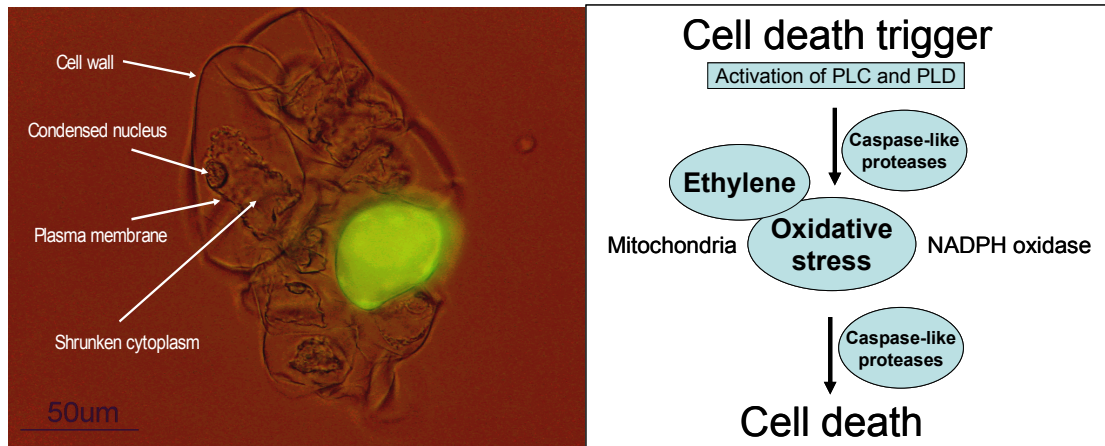


Fig. 1. Cell death in cultured plant cells treated with death-inducing chemicals. Left panel: tomato cell cluster treated with cadmium salt showing fluorescent living cell and non-fluorescent dead cells. Dead cells show shrunken cytoplasm and condensed nuclei. Right panel: Simplified scheme of cell death pathway in suspension cultures cells (see text).



Fig. 2. Storage disorder in apple showing the disappearance of cells.

