Senescence and Programmed Cell Death 30

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Both plants and animals go through onset and progress of certain processes leading to "aging" which ultimately causes death. **Aging** is defined as a degenerative biological change occurring over a period of time. Plants exhibit wide range of variations in life span, ranging from a week to few to many years. It is a common sight in temperate regions that the color of the leaves changes from green to yellow to orange or red before its final fall from the deciduous trees (Fig. 30.1). Such changes happen during the terminal phase of the life cycle of plants and are referred as senescence. **Senescence** is a self-digesting (autocatalytic) process controlled by environment and the genetic makeup of an organism. Changes taking place during this process are catabolic and thus irreversibly degenerative. Senescence is not just a passive decay of structural and biochemical machinery of cells; rather it is a precisely regulated series of events in which organelles, membranes, and macromolecules are broken down. Nutrients, like amino acids, sugars, and minerals, are reclaimed for export out of the senescing organ to other plant parts for later use. Nature is thus conservative as far as its precious resources are concerned. Another general term which is used for mechanisms underlying terminal events in the lives of a plant is **programmed cell death (PCD)**. PCD is also a genetically determined developmental event which leads to elimination of a cell or cells. Such eliminations determine the final shape and habit of a plant. PCD occurs in a wide range of developmental processes. For example, development of unisexual flowers where cells destined to form male or female parts are selectively deleted (Fig. 30.2). Unlike PCD, senescence is a phase of aging process where metabolic processes are catabolic and eventually terminate in death.

With reference to how plants meet their respective ends, they are categorized into **annuals** (which die off at the end of each season), **biennials** (plants living for two seasons), and **perennials** (which produce fruits year after year). Each kind has its

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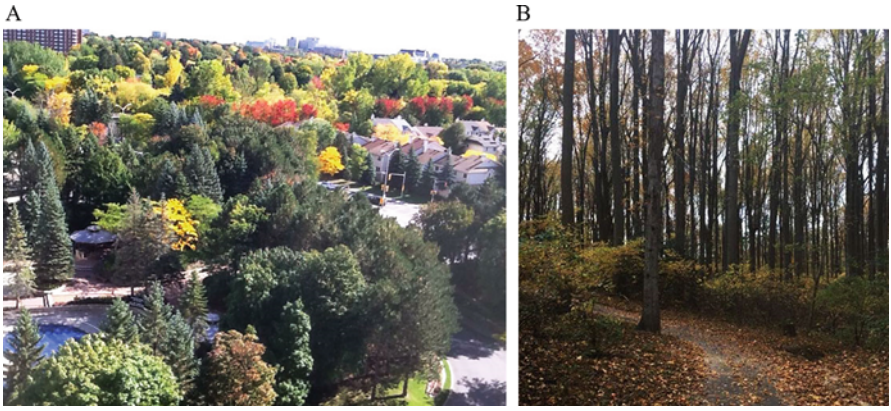

Fig. 30.1 Forest showing green, yellow, orange, and red coloration (**a**) before its final fall from deciduous trees (**b**)

own benefit since annuals result in imparting greater genetic diversity. These plants have better survival strategy because they get new recombinant set of genes every year and they can afford to allocate their energies into producing seeds rather than saving metabolites for the plant to overwinter. On the other hand, perennials can become more robust with each passing year as they add roots, stem, and leaves to already existing structure. Such plants become dominant species in their habitat, and they tend to compete with other plants for light, water, nutrients, and space. Senescence-related changes in annuals occur during leaf maturation phase, and symptoms like abscission appear first in leaves. In evergreen perennials, leaves senesce and abscise after 2–3 years, but not at the same time. There are different factors and mechanisms which govern the senescence of annuals and perennials to be discussed later in the chapter. Senescence is also observed under adverse environmental conditions, like drought, nutrient deficiency, low and high temperatures, but such changes can be reversed when the conditions become congenial unlike natural senescence which is a part of the developmental program.

30.1 Patterns of Senescence

Senescence in plants shows wide range of patterns. Some plants are subjected to death of all aboveground parts at the end of growing season (**top senescence**) or loss of entire leafy cover leaving the stem and roots bare for stipulated time period (**deciduous senescence**); and still another form is the die back of oldest leaves in the normal development of annual plants (**progressive senescence**).

Senescence can be discussed under the following levels in plants.

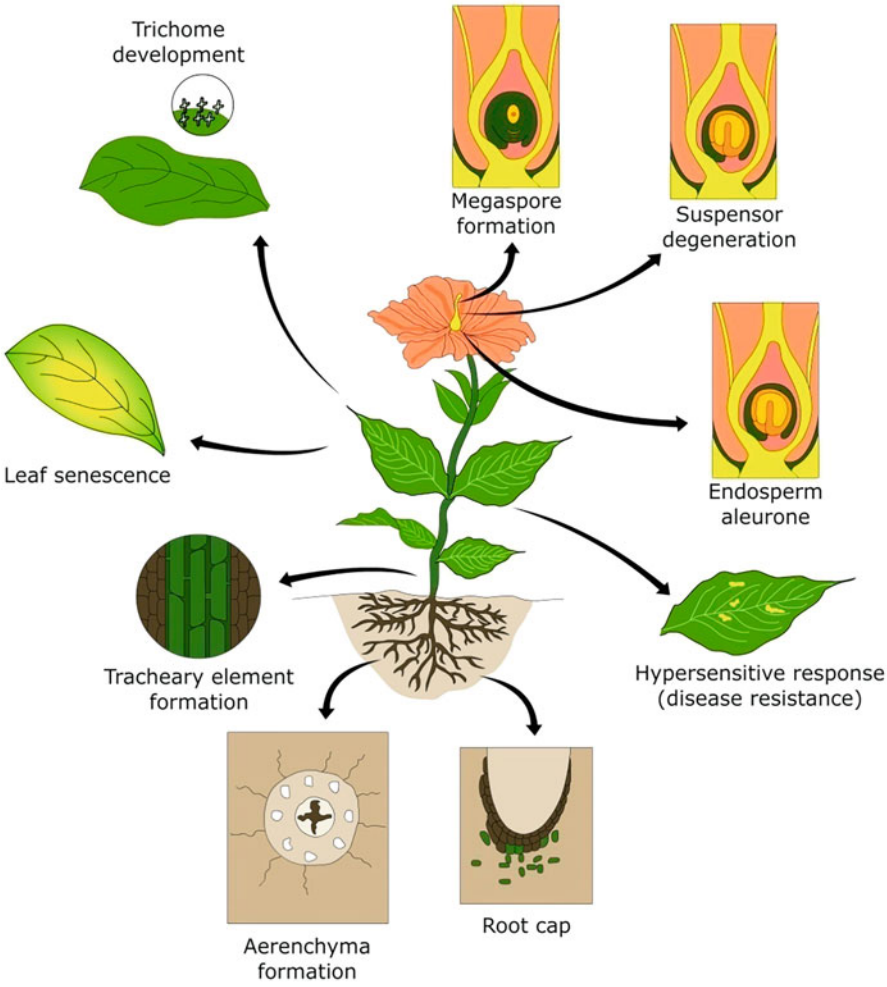

Fig. 30.2 Range of plant processes occurring through programmed cell death (PCD)

30.1.1 Cellular Senescence

Individual cells or small number of cells of an organism undergo senescence. For example, during gametogenesis and embryo development, orderly sequence of events eliminates certain cells. During female gametophyte formation, three out of four cells produced by meiosis are degenerated. The synergids and the suspensor cells also senesce during embryo growth. Such cell elimination is also referred as PCD or programmed cell death.

30.1.2 Tissue Senescence

At specific developmental stages, large group of cells disintegrate and die. For example, tapetum, which is a layer of cells that surround the developing pollen, is degenerated after the maturation of pollen grains. Through lysogenic process, root cortex cells, pith cells, and even mesophyll cells may differentiate into aerenchyma with air spaces. Senescence in a group of cells at specific locations plays a major role in leaf morphogenesis (Box 30.1). Also, mechanical events like tearing and detachment of branches from the plant cause tissue damage through deprivation of nutrient supply leading to tissue senescence.

Box 30.1: PCD During Differentiation: Interesting Facts

Plants undergo localized cell death to create space for transport and secretion, unlike animals where the cells can migrate (gastrulation) to form specific morphological and anatomical structures. New structures are formed due to disintegration of cells (lysigeny), with or without cell separation (schizogeny). Both lysigeny and schizogeny are responsible for differentiation of secretory ducts, cavities, and canals and also in designing the external and internal structures for efficiency in functioning and better adaptive value. Some of the interesting facts about plant structures formed through PCD are as follows:

1. A combination of lysigenous and schizogenous PCD is responsible for formation of oil glands on the surface of citrus fruits which forms a cavity for essential oils.
2. *Avicennia marina*, commonly known as grey mangrove—belonging to Acanthaceae—forms air spaces due to schizolysigeny.
3. *Toxicodendron* spp. (family Anacardiaceae which contains woody trees, shrubs, and vines, including poison oak and the lacquer tree): The resinous secretory ducts in the phloem of these plants develop schizogenously. All members of the genus produce the skin-irritating oil urushiol, which can cause severe allergic reaction.
4. Formation of spines in cacti, where green stem replaces leaves, and the leaves are reduced to spines.

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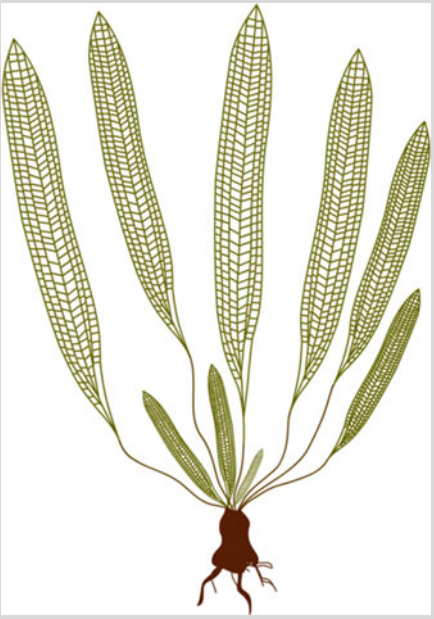
Box 30.1 (continued)



5. *Aponogeton madagascariensis* (commonly known as Madagascar laceleaf or lace plant) undergoes a unique form of leaf sculpting that creates a delicate lattice work. During early development, the lace plant leaf forms a pattern of equidistantly positioned perforations across the surface of the leaf, giving it a lattice-like appearance. Formation of these perforations is a result of PCD, where tonoplast membranes of lace plant ruptures and releases the vacuolar contents. The nuclei retain their identity and DNA becomes fragmented. The walls of the dying cells are also degraded by the action of cellulases and pectinases. After the formation of perforations, the walls of living cells surrounding the perforations get laden by suberin deposition which prevents further spread of PCD and protects the cells from invasion by microbes. Perforations, therefore, are formed at a set time in development and are localized between the interactions of longitudinal and transverse veins across the surface of the leaf. Cell death in this plant occurs at a predictable stage of the leaf development and at a precise location in relation to the completely formed leaf vein system. The lace plant is an ideal system for the study of developmentally regulated PCD in plants.

30.1.3 Organ Senescence

Leaves undergo senescence either on a seasonal basis or in response to environmental stress. Leaf senescence and eventually its fall represents an adaptation either to reduce the water evaporation surface or to allow better light perception by the plant.

30.2 Types of Cell Death

All plants and animals have evolved organized mechanisms for cell destruction which is a part of normal growth and development. PCD can be initiated by developmental signals or by external factors like pathogen attack. In animals, PCD is referred to as **apoptosis**, which is a highly regulated energy-dependent process. This process is characterized by chromatin condensation, nuclear and plasma membrane blebbing, and formation of apoptotic bodies which are eventually engulfed by neighboring phagocytes (Fig. 30.3). Chromosomes also undergo fragmentation as a result of **endonuclease** digestion of DNA between specific nucleosomes. This results in characteristic "laddered" fragments which are multiples of 180 bp. Another set of enzymes called **caspases** (cysteine-dependent aspartate-specific proteases) bring about breakdown of specific proteins which leads to controlled cell death. **Necrosis** is another way of bringing about cell death which generally results from trauma caused by external agents like herbicides. It occurs when the cells are damaged, which results in rupture of membranes, release of cellular contents, and tissue inflammation.

Plant cells differ from animal cells, as they possess cell wall. Some of the normal processes in plants where PCD is observed include degeneration of suspensor during embryo development in angiosperms, survival of one functional megaspore and degeneration of the rest of the three in *Selaginella*, production of unisexual flowers,

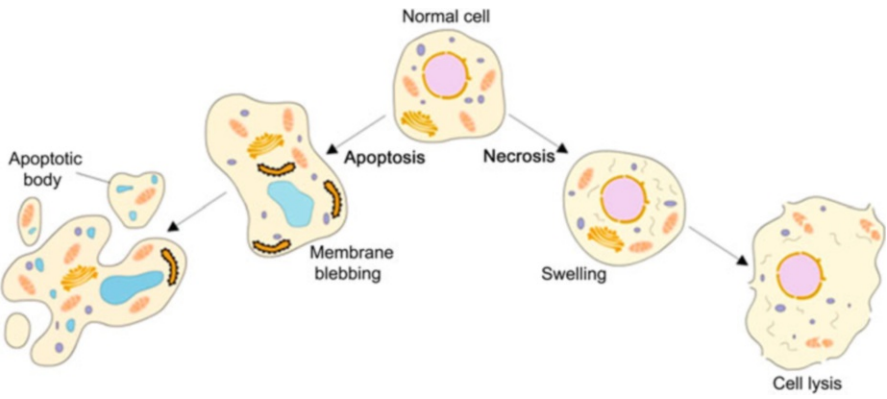


Fig. 30.3 Cell death through apoptosis and necrosis

and differentiation of tracheids and vessel elements in xylem (Fig. 30.2). Two types of PCD pathways have been characterized in plants.

30.2.1 Vacuolar-Type PCD

This process is instrumental in processes like differentiation of xylem elements, leaf senescence, and megasporogenesis. During this process, the vacuole swells and ruptures thereby releasing hydrolases into the cytosol. This release causes breakdown of plasma membrane and complete or partial degradation of cell wall.

30.2.2 Hypersensitive Response-Type PCD

This gets activated in response to pathogen attack wherein the cells at the infection site isolate themselves and bring about self-degradation. This deprives the pathogen of much required nutrients and also checks its further spread. This process is characterized by vacuolar water loss and cell shrinkage, followed by degradation of nuclear DNA (Fig. 30.4). In plants, the general term used for dissolution of cytoplasm within the cell wall through the action of cell's own catabolic machinery is called **autolysis**.

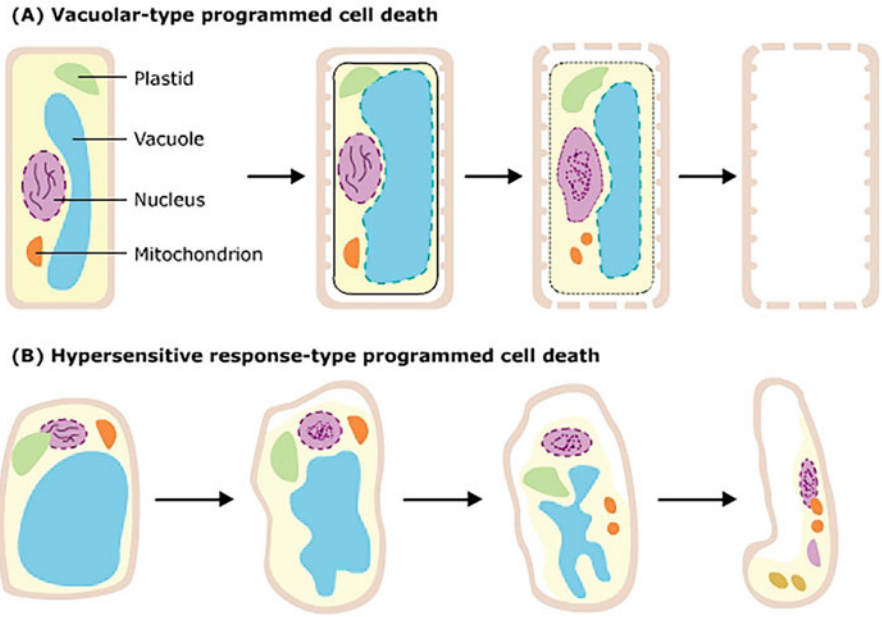


Fig. 30.4 Two types of PCD (**a** and **b**) observed in plants

30.3 Autophagy

Cells have a defined life span, beyond which they are face organism's catabolic activity. Autophagy (self-eating) is one such mechanism whereby cellular components targeted for destruction are collected in lysosomes and degraded within it. Autophagy protects the cell from harmful effects of damaged proteins and organelles (Box 30.2). Moreover, in case of starvation, autophagic breakdown of cell constituents ensures recycling of cellular components which maintain energy levels. During this process vesicles are produced which engulf portions of the cell to be degraded. These vesicles are called **autophagosomes**. The formation of autophagosomes involves well-defined steps:

1. Vesicle induction: a double membrane, cup-shaped structure is pinched off from the endoplasmic reticulum. This is called **phagophore**.
2. Vesicle expansion: phagophore expands and engulfs the components targeted for destruction including the misfolded proteins and aging organelles.
3. Tonoplast docking and fusion: the phagophore becomes spherical after fusion of inner and outer phospholipid bilayer to form complete autophagosome. This fuses with the vacuolar membrane or tonoplast.
4. Digestion: after the fusion, there is generation of single-membrane vesicle called the **autophagic body** which is finally degraded in the vacuole (Fig. 30.5).

Hydrolytic breakdown of autophagic body generates monomers like amino acids and sugars which act as building blocks or energy source for cellular structures. The formation of autophagosomes is regulated by genes which are called **autophagy-related genes** or **ATG genes**. These genes are best described in yeast (*Saccharomyces cerevisiae*) after nutrient starvation and also in *Arabidopsis*. Specific proteins are localized on **phagophore assembly site** of the ER which play a major role in the initiation and growth of autophagosomes. A protein designated as ATG9 moves between phagophore assembly site and *trans*-Golgi network and supplies membrane components to the expanding phagophore. The movement of ATG9 is facilitated by ATG1/ATG13 complex and phosphatidylinositol 3-OH kinase complex. Autophagy regulation has been further understood with the identification of **TOR** (**target of rapamycin**). TOR is a master switch which controls ATG genes, and it constitutes a serine/threonine protein kinase. It acts as a negative regulator of autophagy by phosphorylating the ATG1/ATG13 complex. TOR prevents the binding of ATG1/ATG13 protein complex to **phagophore assembly site** (**PAS**). Due to this, ATG9 fails to obtain fresh membrane lipids for phagophore expansion, thereby inhibiting autophagy. TOR activity, in turn, is negatively regulated by nutrient limitation and other stress factors. Thus, stress can control autophagy by inhibiting TOR activity (Fig. 30.6). In general, plants exhibit accelerated senescence when autophagy-specific genes are silenced (demonstrated in *Arabidopsis*). Autophagy serves as a homeostatic mechanism to maintain the metabolic and structural integrity of the cell.

Box 30.2: Deciphering the mechanism of autophagy bags Nobel Prize

The 2016 Nobel Prize in Physiology or Medicine has been awarded to the Japanese cell biologist Yoshinori Ohsumi, a renowned name in the field of autophagy, for his commendable discoveries leading to understanding the intricacies of the process of cellular "self-eating" or "autophagy." **Autophagy** (derived from the Greek word "autóphagos" meaning "self-eating") refers to a highly regulated process of digestion of obsolete, dysfunctional, or damaged cellular components and recycling of macromolecules for renewal of cellular contents. This process essentially involves sequestration of portions of cytoplasm into specialized saclike membranous organelles called **autophagosomes**. The internalized contents are then delivered to the lysosomes or vacuoles through fusion where their degradation is carried out. It serves as a quality control mechanism that degrades nonessential cellular contents and detoxifies harmful materials. It plays vital roles during different stages of differentiation in a variety of tissues and provides a means to adapt to starvation or stressful conditions. First inkling toward uncovering this pathway of degradation of cellular constituents came in the 1950s with the discovery of specialized compartments in the cell which supposedly harbor enzymes that digest proteins, carbohydrates, and lipids. These distinct membranous structures were given the name **lysosomes** by Christian de Duve in 1955, and for this discovery, he was awarded a Nobel Prize in Physiology or Medicine in 1974. Soon thereafter electron microscopic studies carried out in the 1960s revealed the sequestration of portions of the cytoplasm, including organelles, into small vesicles, which were in various degrees of disintegration. It was then realized that these vesicles had the capacity to digest the internalized contents, and the term "autophagy" was coined by de Duve in 1963 to describe the process of degradation of the internalized contents in these sacs, and the sequestering organelle was named **autophagosome**. de Duve suggested a strong possibility of involvement of this mechanism in the targeted degradation of aberrant and redundant cellular contents. Thereafter, during the period from the 1970s to 1980s, constant efforts were made in explaining the working of the autophagic system. Around the same time, extensive work was being done in discerning another pathway that mediates intracellular protein degradation which eventually leads to characterization of the ATP-dependent **ubiquitin-mediated protein degradation** system via **proteasome** which is now recognized as a major pathway for efficient breakdown of most short-lived proteins. For their outstanding discovery of the ubiquitin-proteasome pathway, Aaron Ciechanover, Avram Hershko, and Irwin Rose were awarded the 2004 Nobel Prize in Chemistry. It was only in the 1990s when Yoshinori Ohsumi's group documented the identification of the gene ***ATG1*** **(autophagy-related gene 1)** in yeast, which the beginning of the molecular era in the field of autophagy was marked, an area which gained significant prominence in the forthcoming years.

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Box 30.2 (continued)

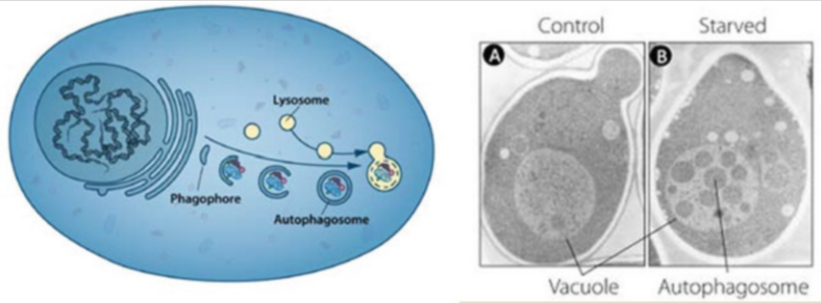


Figure 1: The cells harbor a machinery to dispose off redundant macromolecules and organelles via lysosomal degradation. In plant and yeast cells, vacuole corresponds to lysosomes in animal cells. Parts of cytoplasm are engulfed within the vesicles, known as autophagosomes, which then fuse with lysosome/vacuole, where the contents are degraded into smaller constituents (left panel). Prof. Ohsumi's experiments focused on generating mutant yeast cells lacking vacuolar degradation enzymes and demonstrated the accumulation of autophagosomes in the vacuole in starved yeast cells, thereby indicating the existence of autophagy (right panel)

Prof. Ohsumi's work centered around deciphering the progression of autophagy through different phases by simulating starvation in yeast cells, a condition already known to trigger autophagy. He also elucidated a similarity between the autophagy morphology in yeasts and mammals. By managing to disrupt the degradation process in the vacuole of the mutant yeast cells lacking vacuolar degradation enzymes, his group was able to demonstrate the accumulation of autophagosomes within the vacuoles, while the process of autophagy was active. A series of cloning experiments in yeast and mammalian cells carried out by Ohsumi's group led to the characterization of 15 key genes involved in this process and elucidation of the function of each of the encoded products in promoting distinct stages of autophagosome formation. These momentous investigations undertaken in yeast triggered subsequent analysis of autophagy in higher eukaryotes and have helped in the recognition and establishment of autophagy as a fundamental process in cell physiology. It is now established that the process of autophagy is an evolutionarily conserved process of recycling cellular constituents occurring ubiquitously in eukaryotic cells. Prof. Ohsumi's pioneering work has paved way for development of rational approaches to use autophagy in treating a number of neurodegenerative diseases and cancer effectively.

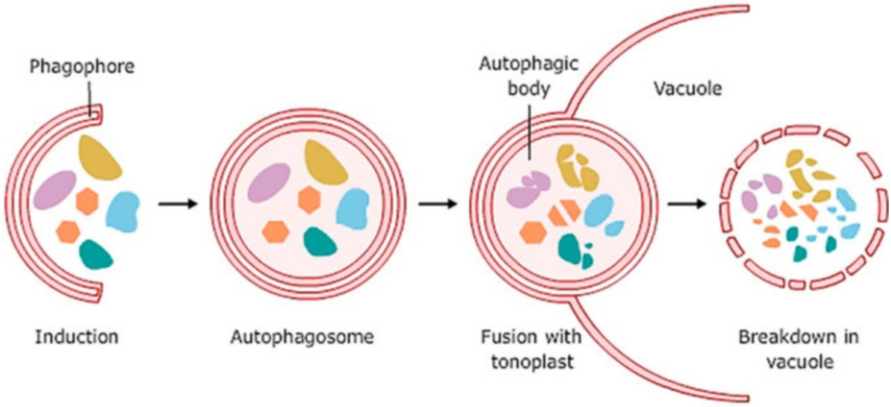


Fig. 30.5 Steps involved in autophagy

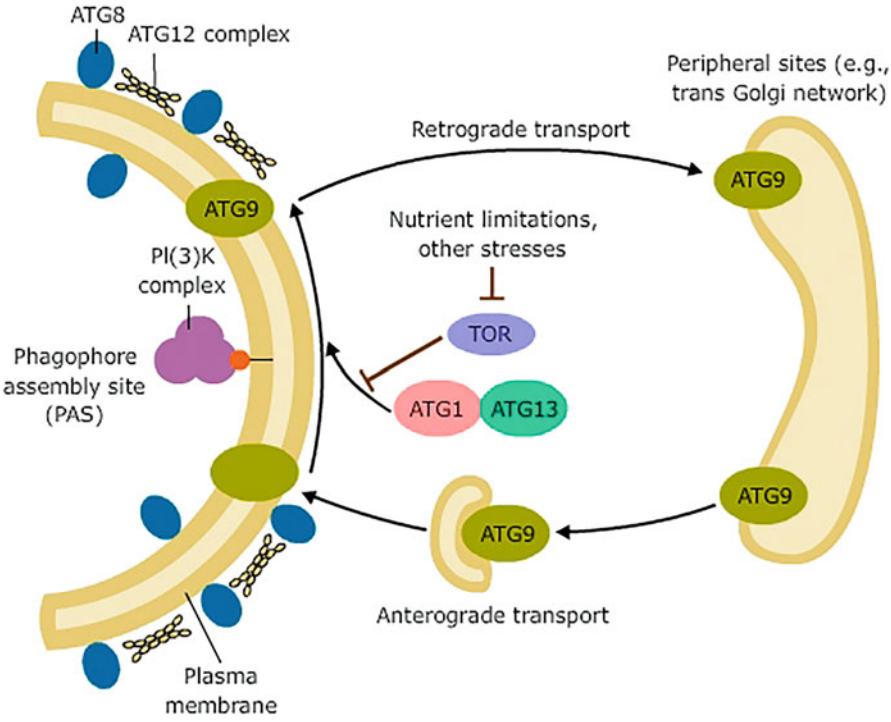


Fig. 30.6 Scheme for autophagy. ATG9 drives the growth of phagophore by shuttling between phagophore assembly site and peripheral membrane sites. ATG9 is facilitated by ATG1/ATG13 and phosphatidylinositol 3-OH kinase complex. TOR promotes the negative regulation of autophagy by phosphorylating ATG1/ATG13 complex, thereby preventing their binding to phagophore assembly site (PAS). ATG, autophagy-related gene; TOR, target of rapamycin; PAS, phagophore assembly site

30.4 PCD During Seed Development

PCD is a fascinating process which remodels the plant at cellular, tissue, and organ level in varied processes such as tracheary element differentiation, lysigenous aerenchyma formation, development of functionally unisexual flowers from bisexual floral primordial, and leaf morphogenesis. Plant development begins from seed which is a highly dehydrated, quiescent, and metabolically slow unit of dispersal. It is a product of double fertilization and constitutes endosperm and embryo. Seeds where endosperm is retained till maturity are termed as endospermic seeds (e.g., *Ricinus*), whereas in non-endospermic seeds, endosperm is consumed by the developing embryo (e.g., *Pisum*). In cereals, endosperm consists of two cell types: the starchy endosperm and aleurone. Both these cell types undergo PCD through distinct routes. The contents of starchy endosperm get desiccated and are later degraded at the time of germination by hydrolytic enzymes, whereas the cells of aleurone layer remain alive until germination and endosperm mobilization. In maize, a mutant called *shrunken2* (*sh2*) has been identified which shows altered endosperm development and premature cell death (Fig. 30.7). Interestingly, ethylene production is higher in kernels of *sh2* mutants. This shows that ethylene plays an important role in regulating PCD in maize kernels. On the other hand, aleurone layer autolysis is regulated by gibberellic acid (GA) and abscisic acid (ABA) (Fig. 30.8). GA stimulates PCD in barley aleurone layer, while ABA delays it. GA-induced synthesis and secretion of hydrolases is also known to be influenced by cytosolic free Ca^{2+}, pH, cyclic GMP, protein phosphatases, and protein kinases. A block in cGMP linked GA-signaling pathway prevents cell death, indicating a promising role for cGMP in signal transduction pathway that leads to cell death in aleurone layer. Nitric oxide (NO) also participates in cereal aleurone PCD. NO donor, like sodium nitroprusside, delays GA-induced cell death, while NO scavengers accelerate the process. NO is an antioxidant and reacts with heme-containing proteins, like guanylyl cyclase required to generate cGMP. NO also nitrosylates proteins containing exposed thiol groups, causing reversible conformational change.

30.5 PCD During Tracheary Element Differentiation

Water and minerals conducting strands of plants, the xylem, contain tracheary elements (TEs) and vessel elements. Differentiation of tracheary elements includes secondary cell wall thickening, followed by autolysis of protoplasm, and cell death. Breakdown of protoplasm is accompanied by an increase in the activity of degradative enzymes, including DNases, RNases, and proteases (Fig. 30.9). Differentiation of isolated *Zinnia elegans* mesophyll cells is used to analyze process of tracheary element differentiation. In *Zinnia* cell cultures, individual mesophyll cells have been shown to transdifferentiate directly into tracheary elements without cell division, in the presence of phytohormones. Cell wall undergoes lignification, and all cell organelles degenerate to provide a clear passage for water movement. Calcium and calmodulin (along with auxins and cytokinins) also play a role in tracheary element

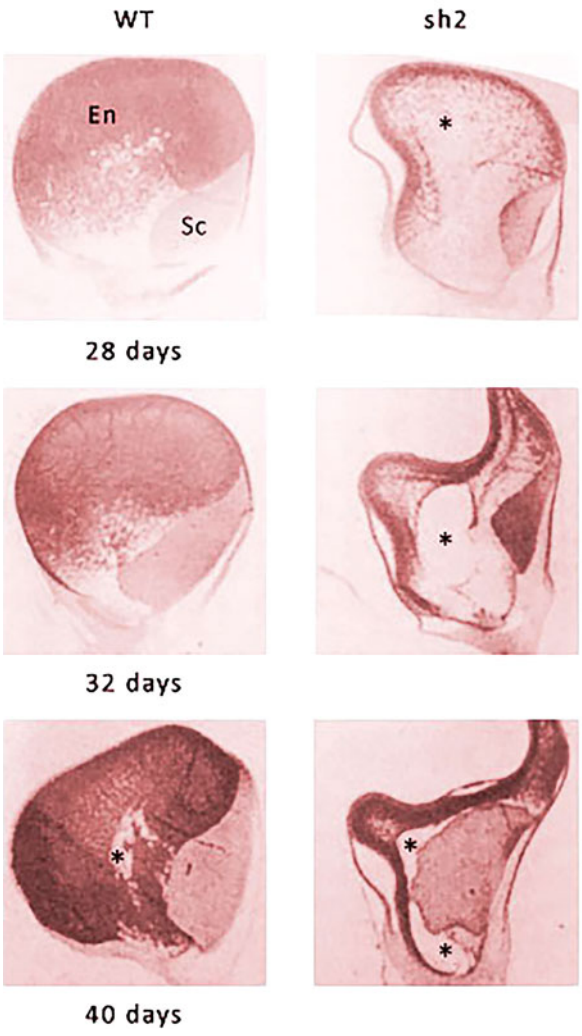


Fig. 30.7 PCD during development of maize aleurone layer. Mutant*, *shrunken 2 (sh2)* shows altered endosperm development and premature cell death as compared to the wild type (WT). En, endosperm; Sc, scutellum

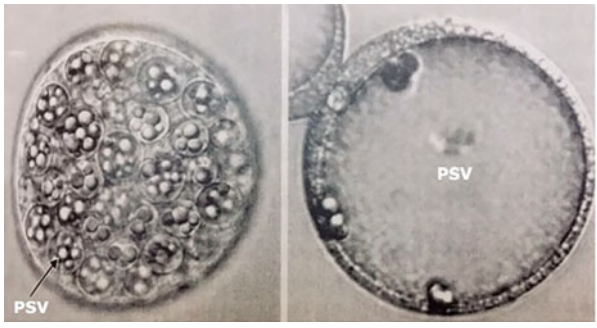


Fig. 30.8 PCD in cereal aleurone showing vacuolation of cytoplasm without rupture of membranes. Membrane integrity is maintained until cell death. PSV, protein storage vacuole

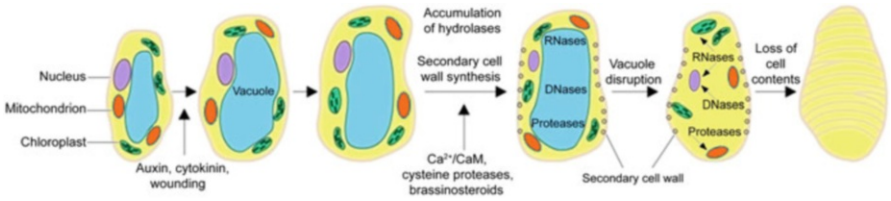


Fig. 30.9 PCD during tracheary element (TE) differentiation. Auxins, cytokinins, and calcium play a role in TE differentiation

differentiation. Removal of calcium from the cultures and administration of calcium channel blockers inhibit the differentiation process.

30.6 PCD During Gametogenesis

During the development of unisexual flowers, either male or female parts are eliminated via PCD. For example, in maize, cell death takes place during the formation of unisexual male and female flowers during gametogenesis. The genetic control of this process has been investigated by analyzing mutants. Young maize flowers in the tassel contain primordia for both male and female flowers, but gynoecium cells stop growing during the course of development. In *tasselseed2* mutants, arrest and degeneration of gynoecia do not occur, and female flowers are produced in the tassel. Thus, dominant mutant, TASSELSEED2 (TS2) is required for the death of developing female organs in tassels. With the expression of TS2, gynoecial degeneration starts. TS2 encodes hydroxysteroid dehydrogenase, which might regulate cell death through a steroid-like molecule which acts as a switch in cell death pathway. TS2 is not expressed in female spikelet as it is suppressed by another gene SILKLOSS1(SK1). Both TS2 and SK1 selectively promote or inhibit cell death in maize. In angiosperms, three out of four megaspores formed after meiosis undergo PCD. Tapetum tissue also disintegrates at the time of pollen formation, showing symptoms like cell shrinkage, condensation of chromatin, swelling of ER, and persistence of mitochondria. Timing of cell death is also important as inviable pollen is formed when tapetum undergoes early PCD and pollen abortion occurs in case tapetal cell death is blocked.

30.7 Leaf Senescence

Leaf senescence is a final stage of development which involves nutrient relocation from the leaf to growing vegetative or reproductive parts of a plant. It involves coordinated action at cellular, tissue, organ, and organism levels. Our understanding regarding molecular basis of leaf senescence has come through characterization of senescence mutants and **senescence-associated genes (SAGs)**. Leaf cells undergo genetically programmed changes in cell structure and metabolism. It is thus regarded

as a highly regulated ordered series of events where organelles, membranes, and macromolecules are broken down and nutrients like amino acids, sugars, and minerals are reclaimed from the leaf to be reused in other parts of the plants. Senescence in grains, like wheat or barley, is quite characteristic, i.e., the entire resources of the plant are mobilized and reclaimed to support grain or seed development. First symptom of senescence is a decline of photosynthesis and increased rate of respiration. Other changes are breakdown of chloroplast membranes, loss of chlorophyll, and protein and lipid metabolism. It is important for senescence that certain cell organelles and tissues remain intact and functional until after mobilization is completed. Hence, they are degraded in certain order. Chloroplasts are rich reservoirs of proteins (like Rubisco, chlorophyll a-/b-binding proteins) and membrane lipids. They are degraded first, while mitochondria, peroxisomes, and nuclei remain functional and transcriptionally active till late stages of senescence. Guard cells in the epidermis and phloem tissue also remain functional for effective gaseous exchange and transport until the metabolic breakdown of chloroplasts and export of metabolites is completed. Also, for intercellular traffic and movement of materials in and out of vacuoles, it is important that the plasma membrane and vacuolar membrane remain functional and selectively permeable till the end. Further, senescence is not altogether a degradative process. Many proteins are synthesized during the course of this terminal plant process, like those associated with synthesis of anthocyanins and carotenoids, which are formed after the loss of chlorophyll pigment. Leaf senescence is governed by the age of the plant under normal growth conditions, which takes place from the most mature to the youngest leaves (sequential leaf senescence). It may also take place in response to climate change, as in deciduous trees (seasonal leaf senescence). Both these types of senescence are part of developmental leaf senescence which consists of three distinct phases: **(1) Initiation phase**: at this stage, the decline in rate of photosynthesis takes place. **Blebbing**, a process that occurs in healthy cells, stops in senescing cells. In this process, membrane turnover takes place by the removal of lipid metabolites by forming lipid-protein particles which are shed by blebbing. This process stops during senescence. As a result lipid-protein particles accumulate between lipid bilayers, causing the membrane to become leaky. **(2) Degenerative phase**: this phase is marked by autolysis of cellular organelles and macromolecules. Many new genes are expressed, while those involved in photosynthetic activity are "turned off." **(3) Terminal phase**: during this phase, autolysis is completed, and cell separation takes place at the abscission layer (Table 30.1). All these phases merge to bring about leaf senescence by massive reprogramming of gene expression.

During chloroplast degradation, its primary degenerative products are extremely photoreactive and prove lethal to the cell. Thus, it is important to safely remove and dispose these toxic compounds. Thus, chloroplasts are transformed into **gerontoplasts** (a term introduced to define the unique features of plastids formed during leaf senescence). The formation of gerontoplasts from chloroplasts during senescence involves extensive structural modifications of thylakoid membrane with simultaneous formation of a large number of plastoglobules with lipophilic materials. The structural dismantling of grana is accompanied by a decline in

Table 30.1 Phases of leaf senescence and accompanying events/factors

Phase	Factor/event
Initiation phase	**Internal factors**: sugars, phase change, hormones (auxin, cytokinins, salicylic acid, jasmonic acid, ethylene, abscisic acid)
	External factors: shading, heat or cold, pathogen attack or wounding, UV or ozone, drought, nutrient limitation
Degenerative phase	**Cell degeneration**: salvage and translocation of nutrients (e.g., nitrogen and lipids), detoxification and defense (e.g., antioxidant production and activation of defense-related genes), chlorophyll loss, macromolecule degradation
Terminal phase	**Cell death**: disruption of nucleus and mitochondria, DNA laddering, breakdown of plasma and vacuolar membranes

primary photochemical reactions. Gerontoplasts retain their ability to divide, and their development is reversible up to a certain threshold. This ability is lost as the cells enter the terminal phase of senescence. Chlorophyll breakdown proceeds by the loss of phytol tail, which is cleaved by a chlorophyllase action to yield chlorophyllide. Magnesium is removed from the porphyrin ring by an enzyme (magnesium dechelatase) that dechelates it. The resulting pheophorbide is degraded further in a two-step process to give rise to a colorless, straight-chain tetrapyrrole, and the chlorophyll-binding proteins are released for degradation. The remaining chlorophyll catabolites are exported from the chloroplast, modified slightly in the cytosol, and then imported into the vacuole, where further degradation occurs. Studies have demonstrated that the autophagy pathway is required for whole chloroplast breakdown during dark-induced leaf senescence.

A number of cDNAs have been cloned from plants like *Arabidopsis*, asparagus, maize, tomato, etc. during senescence. These are the genes whose expression is upregulated during senescence and are called **SAGs (senescence-associated genes)**. These genes are divided into two classes: (1) those which are expressed at a low basal level throughout most of the leaf development, but their expression is upregulated with the onset of senescence, and (2) those which are expressed only with the onset of senescence. Some of these genes are associated with biotic and abiotic stress conditions, response to reactive oxygen species (ROS), metal-ion binding, pectin esterase, and genes involved in lipid mobilization. Nitrogen is also translocated in phloem stream mainly in the form of amides, glutamine, and asparagine. Glutamine synthases (GS) are the enzymes that convert ammonia to glutamine. Two types of GS occur in plants. GS1 is located in cytosol, while GS2 is located in plastids. During senescence, the activity of GS2 decreases while that of GS1 increases. Several genes encoding GS1 are upregulated which suggests that ammonia released from the catabolism of amino acids may be reconverted to glutamine in the cytoplasm for transport. Further, gene coding for phosphoenolpyruvate carboxykinase (PEPCK), which converts oxaloacetate to PEP (a step in gluconeogenesis), is also upregulated. Genes encoding phospholipase D and a β-galactosidase, enzymes that are involved in the hydrolysis of membrane phospholipids and galactolipids, are also upregulated. Genes whose expression is suppressed by senescence are called **SDGs (senescence-downregulated genes)**.

Table 30.2 Metabolic pathways upregulated/downregulated during senescence in *Arabidopsis*

Upregulated genes	Downregulated genes
Autophagy transport	Amino acid metabolism
Response to ROS	Chlorophyll biosynthesis
ABA signaling	Carotenoid biosynthesis
Metal-ion binding	Cytokinin-mediated signaling
DNA binding	Glycine metabolism
Protein binding	Photosynthesis
Carotene metabolism	Glutamine synthase 2
Caspase activity	
Pectinesterase activity	
Ethylene signaling	
Lipid catabolism	
Glutamine synthase 1	

Table 30.2 depicts the list of metabolic pathways that are either upregulated or downregulated during senescence in *Arabidopsis*.

30.8 Hormonal Regulation of Senescence

Senescence is regulated by the developmental program of the plant, but it can be modulated by several hormones. It ensures efficient remobilization of nutrients to vegetative or reproductive sinks. Gibberellins, cytokinins, and brassinosteroids retard senescence, while ethylene, ABA, and jasmonates enhance senescence-related changes. However, it is observed that the same hormone can act as positive or negative regulator of senescence depending on the age of the leaf. At the same time, leaves should also develop competence to senesce, before they can respond to positive senescence regulators. There is a crosstalk between hormones which regulates leaf senescence, depicting a web of control mechanisms.

30.8.1 Cytokinins

They are known to delay senescence but do not prevent it indefinitely. It is observed that local application of cytokinin to mature green leaves delays senescence in the area where cytokinin is applied, while the rest of the leaf shows senescence. If *Arabidopsis* or tobacco plants are transformed with *ipt* gene from *Agrobacterium*, which encodes an isopentenyl transferase (this gene leads to an overproduction of cytokinin), the transformed plants show delayed senescence (Fig. 30.10). Gene transcripts involved in cytokinin biosynthesis decline, while transcripts of genes involved in degradation of cytokinins (like cytokinin oxidase) increase during senescence. These results suggest that cytokinins are natural regulators of senescence. The AHK3 is the receptor that regulates leaf senescence in *Arabidopsis*. Elevated levels of this receptor result in delay of senescence, while its disruption leads to premature leaf senescence. When *ipt* gene

Fig. 30.10 Progressive loss of chlorophyll during tobacco leaf senescence. Note that the last areas to lose the green color are close to the veins, reflecting the fact that cells close to the veins need to remain active during nutrient export (*Contributed by: Neha Singh*)

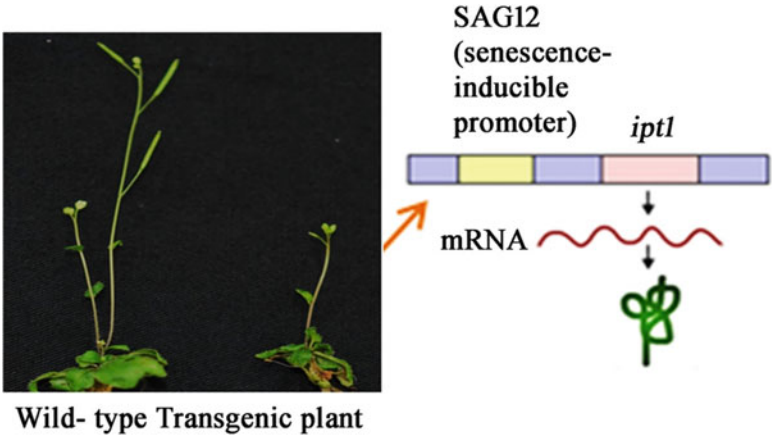


Fig. 30.11 Plants transformed with *ipt* gene leads to delayed senescence due to overproduction of isopentenyl transferase

is fused to the promoter of SAG12 (a gene specifically expressed in senescing tissues), it results in expression of the transgene at the time of senescence (Fig. 30.11). Cytokinin production is autoregulated as its production inhibits senescence, and this results in decreased expression of the transgene. In these plants, older leaves retain the ability to photosynthesize much longer than the leaves of control plants lacking the transgene. Such plants show significant increase in seed production and improved

drought tolerance. It is believed that cytokinin represses leaf senescence by regulating nutrient mobilization and through regulation of sucrose levels. It also imparts enhanced sink strength to the organ concerned. Also, cytokinins are transported from roots to leaves. It is suggested that after flowering, cytokinins are redirected from roots to developing seeds instead of leaves, and this is instrumental in triggering leaf senescence. Cytokinin may also repress expression of key SAGs. For example, genes encoding a cysteine protease and a peroxidase are upregulated in *Petunia hybrida* callus cultures when transferred to low cytokinin medium. It is evident that cytokinins act at two levels: at a distance by promoting differentiation and strong sink activity and locally in senescing cells by mediating the initiation of senescence program. A very interesting action of cytokinin is seen during regreening of tobacco plants. The oldest leaf of *Nicotiana rustica* which has lost all chlorophyll can recover full greenness by removal of stem at the node or by treatment with benzylaminopurine (a synthetic cytokinin). Such leaf can differentiate its gerontoplasts into functional chloroplasts. Proteins characteristic of chloroplasts assembly in very young cells, like chlorophyll synthesis enzyme-protochlorophyllide oxidoreductase, are re-expressed (Fig. 30.11). This points at the fact that cytokinin is a key factor in delaying senescence, and also senescence is a reversible phenomenon even at an advanced stage.

30.8.2 Auxin

High auxin concentrations enhance ethylene production, which in turn, promote senescence in mature leaves. The level of auxin increases during leaf senescence, and many genes involved in auxin biosynthesis, such as tryptophan synthase, are upregulated during senescence. However, application of exogenous auxin in *Arabidopsis* leads to a decrease in expression of SAGs. Overexpression of YUCCA6, the flavin-containing monooxygenase that catalyzes the rate limiting step in auxin biosynthesis, delays leaf senescence and decreases SAG expression.

30.8.3 Gibberellins

Gibberellins play an important role in senescence as their active forms are known to decline in leaves with progressing age. The addition of GA_3 to leaf discs of *Taraxacum officinale* retards their senescence and delays decline in the levels of chlorophyll, protein, and RNA. Incorporation of radioactive leucine and adenine into protein and RNA, respectively, is increased by GA. This enhancement of protein and RNA synthesis does not occur if the discs are supplied with actinomycin D before treatment with gibberellin. However, if actinomycin D is added after the gibberellin treatment, then the stimulatory effect of the hormone is maintained. These results suggest that the retarding action of gibberellins on leaf senescence could be mediated through regulation of RNA synthesis, which is DNA dependent. In another experiment, gibberellic acid (at concentration 10^{-5} and 10^{-4} M) delayed the senescence of carnation flower, when applied continuously to flowers between the closed brush

and fully open stages of development. Treatment with paclobutrazol, an inhibitor of GA biosynthesis, prevented tight buds from opening fully, reduced the longevity of partially open flowers, but was ineffective when applied to fully open flowers. GA-treated flowers did not show simultaneous petal in-rolling, a known indicator of senescence, and the time to complete petal drying was extended. These results provide evidence for a positive role for gibberellins in development of a flower and delaying senescence.

30.8.4 Jasmonic Acid

Jasmonates (JA) are oxylipins derived from linolenic acid in chloroplasts membranes. These hormones have been shown to reduce the photochemical efficiency of photosystem II and the levels of chlorophyll content of detached leaves when applied exogenously. A gene which is involved with senescence and jasmonic acid receptor in *Arabidopsis* has been designated as **coronatine-insensitive 1 (COI1)**. JA treatment accelerated leaf senescence in wild-type *Arabidopsis* plants but not in *coi1* mutants. Exogenous application of JA stimulates leaf senescence and controls the expression of a series of senescence-related genes.

30.8.5 Abscisic Acid

It promotes senescence and abscission of leaves, and the levels of the hormone increase dramatically in leaves that are undergoing senescence. Some of the key ABA biosynthesis genes are upregulated at the time of senescence, like NECD and aldehyde oxidase genes. It has been put forward that ABA is able to increase H_2O_2 levels (either by production or accumulation) in leaves, and this accelerates senescence process. Role of ABA in the regulation of senescence has been investigated in detached tobacco leaves (*Nicotiana rustica*). Leaves which senesce in darkness show a sharp rise in ABA level in the early stage of aging, followed by a rapid decline. The same trend is found when leaves are aged in light, but the rise in ABA occurs 4 days later than in darkness. Senescence is slower in light than in darkness. On the other hand, leaves treated with kinetin which senesce in light and darkness do not show an increase in ABA. Application of kinetin leads to a transformation from free to bound ABA. These results indicate that ABA and cytokinins are involved in a trigger mechanism to regulate senescence. The stage at which this trigger is activated determines the rate of senescence. ABA levels are also observed to rise under water or salt stress conditions. Senescing leaves dehydrate more rapidly than normal leaf because ABA-induced stomatal closure stops functioning. In senescing leaves, stomata stay open due to SAG113 gene which is induced by ABA. This gene encodes protein phosphatase 2C which inhibits stomatal closure in senescing leaves. Loss of function of SAG113 delays senescence, and its overexpression accelerates it. Before the onset of senescence, ABA induces stomatal closure to reduce loss of water. Contrary to this, ABA signaling changes to induce genes like SAG113 which

inhibit ABA-induced stomatal closure and accelerates senescence. This also demonstrates how one hormone can have different effects at different phases of development.

30.8.6 Ethylene

Well known for its involvement in fruit ripening, ethylene is also involved in the control of senescence in leaves and flowers. Exogenous application of ethylene to leaves induces senescence-related changes in leaves and in flower petals. Endogenous ethylene levels are known to increase as the leaves get older, indicating its role in senescence-related changes. The use of inhibitors of ethylene action, such as silver thiosulfate or 1-methyl cyclopropane (1-MCP), prevents senescence of cut flowers and detached leaves. Expression of genes encoding ACC synthase (ACS) or ACC oxidase (ACO) in an antisense orientation in transgenic plants results in delayed senescence, whereas reverse happens if ethylene is overproduced by overexpression of an ACS gene. These effects of ethylene are brought about when the leaves or flowers are at the correct developmental stage, i.e., young leaves or young flower buds do not show senescence-related changes, despite the presence of ethylene. Interestingly, senescence of leaves and ripening of climacteric fruits share some common features, viz., both involve chlorophyll degradation, an increased activity of hydrolytic enzymes, and ethylene production. However, both are different processes, involve different sets of enzymes, and lead to different end results. In many plants (like *Petunia hybrida*), pollination triggers floral senescence, and ethylene production is detected within 20 min of pollination. The recognition of pollen by stigma is the trigger which induces ethylene production. Further evidence for the critical role of ethylene in senescence comes from *Arabidopsis etr1* mutants and *never ripe* tomatoes which are insensitive to ethylene due to mutation in an ethylene receptor protein. Both these mutants show delayed senescence, but it is also observed at the same time that leaves of *Arabidopsis* mutants eventually senesce, but tomato mutants never fully ripen. This indicates that ethylene promotes senescence in leaves, whereas it is an absolute requirement for tomato ripening.

30.8.7 Salicylic Acid

This hormone is known to play a role in plant responses to pathogens. It also influences the setting up of kinetics of hypersensitive response and in inducing tolerance for stress. It also plays a role in age-dependent senescence, and the levels of the hormone increase four times in leaves undergoing senescence. In *Arabidopsis* SAG12 and PR1a have been shown to be undetectable when SA is not present in the leaves. Interestingly, transcriptome changes that occur through the presence of SA are similar to those that occur naturally in age-dependent senescence. *Arabidopsis* plants defective in the SA-signaling pathway (*npr1* and *pad4* mutants and *NahG* transgenic plants) have been used to investigate senescence-enhanced gene

expression, and a number of genes show altered gene expression pattern. The presence of SA induces the expression of cysteine protease gene SAG12. Such a change in gene expression delays yellowing and reduces necrosis in the mutant plants defective in SA signaling. This suggests a role for SA in cell death that occurs in final stages of senescence. A crosstalk has also been found between SA and JA with reference to senescence. Senescence-specific WRKY53 transcription factor interacts with the JA-inducible protein EPITHIOSPECIFYING SENESCENCE REGULATOR (ESR/ESP). The expression of these genes is antagonistically regulated in response to JA and SA, respectively, and they influence each other negatively which is most likely governed by the JA and SA equilibrium.

30.9 Developmental Regulation of Senescence

A number of external and internal factors influence various signaling pathways which alter gene expression resulting in sequentially organized leaf senescence. The external factors include seasonal changes and various biotic and abiotic factors, while internal factors include mainly the developmental phase of the plant (age of the leaf). The disposal of leaf is considered as an indirect selection for nutrient salvage. In other words, senescence syndrome is characterized by recruitment of nutrients from leaf tissues which is a part of genome optimization program. Thus, leaf senescence is a consequence of natural selection for genome reproduction and is initiated and progresses in age-dependent manner even if the plant is growing in adequate nutritional conditions away from pathogen attack as well as from biotic and abiotic stresses. Some of the pathways which are activated during leaf senescence include ROS-based signaling, the ubiquitin-proteasome pathway, etc. Epigenetic mechanisms also alter gene expression through histone and DNA modification and chromatin remodeling. Small RNAs also modulate gene expression at posttranscriptional level. The NAC and WRKY genes are the two most abundant families of differentially regulated transcription factors during senescence. NAC transcription factor contains a highly conserved N-terminal DNA-binding domain and a variable regulatory C-terminal domain. NAC gene was first discovered in cereals as a gene regulating senescence. The presence of a functional allele of NAC gene, called NAM-B1, causes earlier leaf senescence and nutrient translocation to the developing grains in a wild variety of wheat. This allows the grains to obtain benefit of reclaimed nutrients from the leaves. In another domesticated variety of wheat, a frameshift mutation results in loss of function of NAM-B1 allele which delays senescence. Early leaf senescence improves nutritional quality of the grain which points at important role of nutrient remobilization during leaf senescence for normal grain development. Another group of transcription factors, designated as WRKY, also plays a regulatory role in promoting leaf senescence. Leaf senescence is delayed in knockout mutants of WRKY53 gene in *Arabidopsis*. Expression of this gene is suppressed by light and promoted by darkness or ROS.

30.10 Role of ROS in Leaf Senescence

The process of senescence and abiotic stress is associated with overproduction of reactive oxygen species (ROS). ROS constitute H_2O_2, superoxide, singlet oxygen, and hydroxyl radicals, which cause oxidative damage to DNA, proteins, and membrane lipids. ROS contribute to progression of leaf senescence as the antioxidant property of the leaf declines. They also act as signals that activate genetically programmed pathways of gene expression that lead to regulated cell death events. ROS generally initiate cell death through lipid peroxidation. In this process hydrophilic moieties are introduced into lipid bilayers, leading to their disruption and the leakage of cytoplasmic contents. Further, degradation of membrane lipids results in free fatty acids which initiate oxidative deterioration by providing a substrate for the enzyme lipoxygenase, causing membrane lipid peroxidation.

30.11 Role of Sugar Accumulation in Leaf Senescence

Sugars are the building blocks for macromolecules and serve as energy source during various metabolic activities. They are now also known to serve as signaling molecules in regulating metabolic events. Investigations have shown that high concentration of sugars lower the photosynthetic activity and may also trigger leaf senescence if they exceed a certain threshold limit. Such sugar-induced leaf senescence is observed under low-nitrogen conditions.

30.12 Role of Pigment Composition in Senescence

Senescence of leaves is also characterized by color change which is related to nutrient mobilization and their reabsorption from leaf cells (Fig. 30.12). Pigment metabolism has adaptive significance as color of flowers and ripe fruits aid in pollination and effective seed dispersal. Pathways of chlorophyll breakdown and anthocyanin biosynthesis are specifically upregulated during senescence. Chlorophyll degradation starts with the release of chlorophyll from its association with pigment-binding proteins in the thylakoid membranes. This process is aided by a gene called **stay green (SGR)**. The gene has a highly conserved structure and is found across all groups of plants like mosses, algae, and prokaryotic cyanobacteria. SGR functions in the disassembly of thylakoid photosystem complexes, making chlorophyll available for degradation. In the next step, the magnesium of chlorophyll a is removed, resulting in production of phaeophytin a (Fig. 30.13). Phaeophytin a is hydrolyzed by the enzyme phaeophytinase to yield pheophorbide a. The other product of phaeophytinase action is phytol, which accumulates in the **plastoglobules** (lipid droplets) of gerontoplasts, largely in the form of esters. Chlorophyll b is converted to chlorophyll a before it can be subjected to breakdown pathway. Chlorophyll b reductase is instrumental in this breakdown and is known to be activated during senescence. Both phaeophytin and phaeophorbide retain

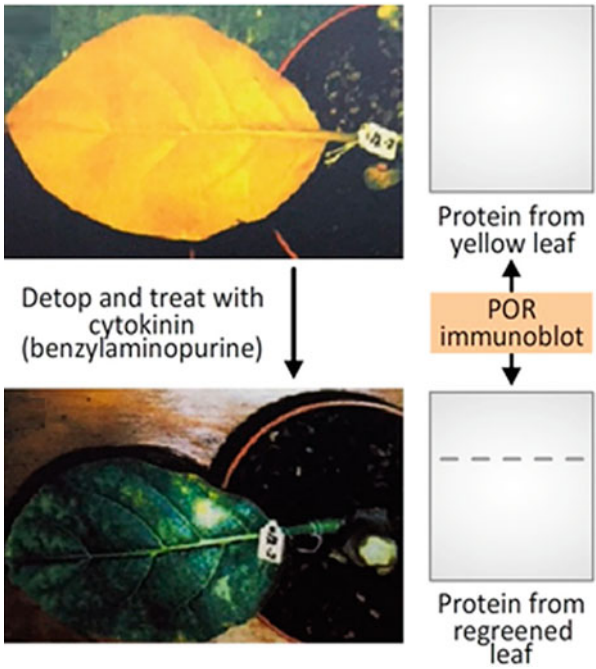
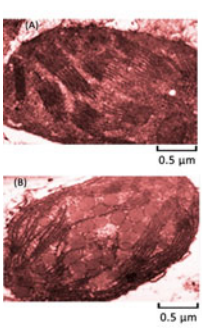


Fig. 30.12 Regreening of tobacco plants by treatment with benzylaminopurine. Proteins characteristic of chloroplast assembly are re-expressed indicating a role of cytokinin in delaying senescence



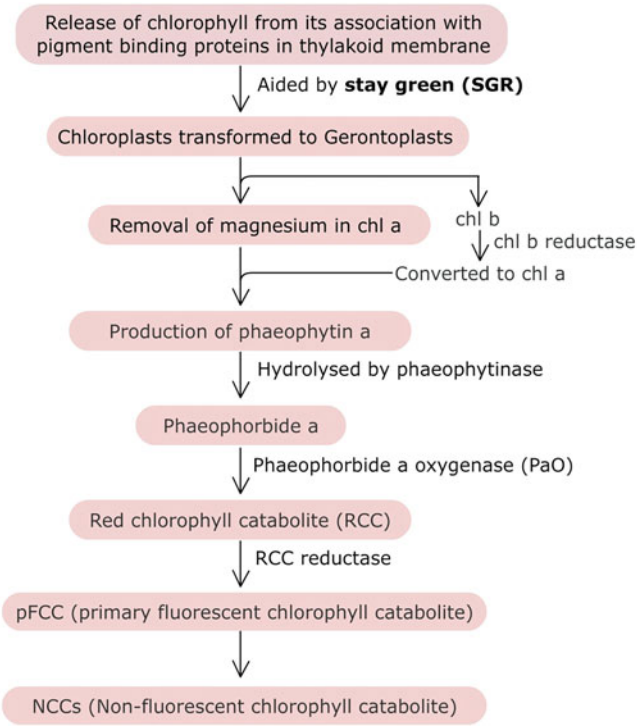


Fig. 30.13 Steps in chlorophyll degradation during senescence

tetrapyrrole ring structure which is later opened to generate a colorless straight-chain tetrapyrrole. Enzyme *phaeophorbidea oxygenase* (PaO) aids this reaction which requires oxygen and Fe, operating in redox cycle driven by reduced ferredoxin. PaO uses pheophorbide a as a substrate which generates a red chlorophyll catabolite (RCC) that does not accumulate in plants but is metabolized further by RCC reductase. This enzyme catalyzes the ferredoxin-dependent reduction of a double bond in the pyrrole system of RCC to produce colorless tetrapyrrole with a strong blue fluorescence (primary fluorescent chlorophyll catabolite, pFCC). FCC is exported from the gerontoplasts to the cytosol by an ATP-dependent transporter located in the plastid envelop and enters the vacuole through ABC transporters in the tonoplasts. pFCC is then modified either by hydroxylation or by conjugation to form nonfluorescent chlorophyll catabolites (NCCs). In this way all carbon and nitrogen in the chlorophyll molecule end up in the vacuole, and chlorophyll molecule is abandoned after dismantling (Fig. 30.14).

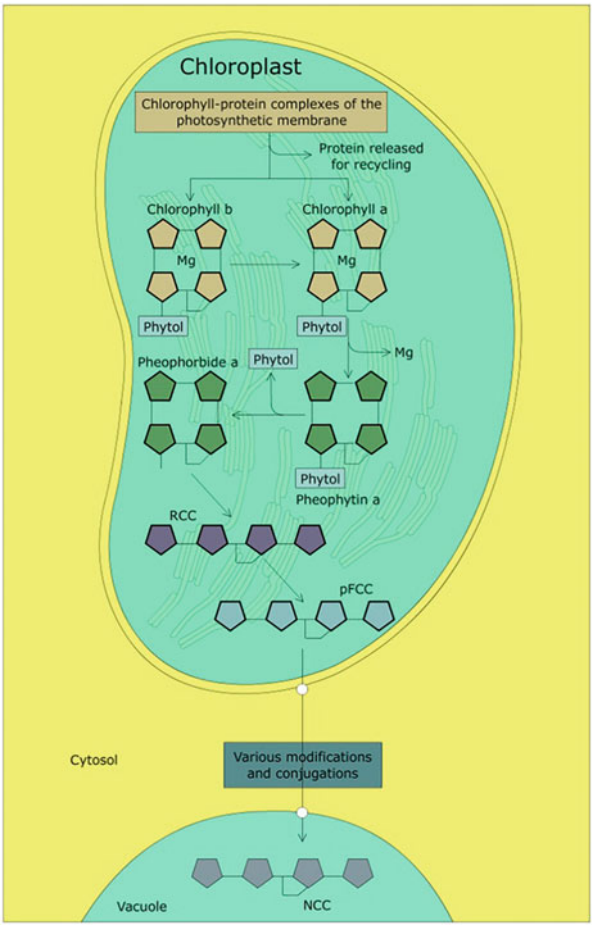


Fig. 30.14 Dismantling of chlorophyll molecule during senescence

30.13 Leaf Abscission

Shedding of leaves in deciduous trees is an intriguing phenomenon, but for plants it is a process by which it gets rid of organs that are no longer required. Abscission usually follows senescence of the organs, but senescence is not essential for an organ to be abscised. The shed organs are generally leaves, flowers, floral parts, mature fruits, etc. It is a controlled process that is initiated in advance of the actual shedding of the organ. An **abscission zone** is formed in a region between the organ to be shed and the body of the plant (Fig. 30.15). This zone facilitates separation by the hydrolysis of wall materials between defined cell layers and to initiate the synthesis of materials that protect the body of the plant from water loss and from infection by microorganisms. Abscission zone can be morphologically identified as one or more layers of isodiametrically flattened cells. Location of abscission zone varies in different organs. For example, in leaves, it is usually formed at the base of the petiole near its junction with the stem. It is characterized as a band of small, densely cytoplasmic cells arranged in rows from 5 to 50 layers in thickness. Before abscission, a **separation layer** is formed within the abscission zone. Cells in the separation layer synthesize and secrete wall hydrolases between the two layers of cells, thus dissolving the middle lamella and disrupting the primary wall. The fracture occurs between the two layers across the width of the petiole. All cells of the layer

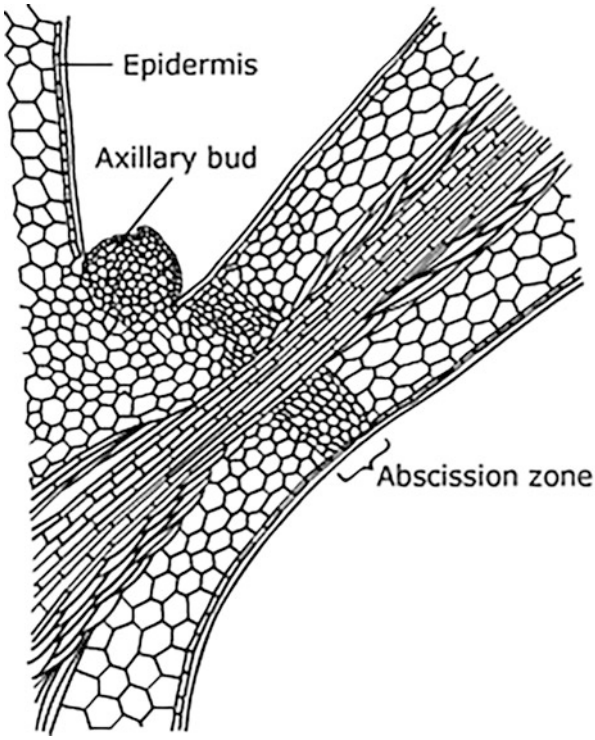


Fig. 30.15 Formation of abscission zone in the leaf axil

participate in the separation except the dead tracheary cells and vessel elements which are broken mechanically. Later, broken vascular elements are plugged with tyloses or gums. A few layers of cells on the proximal side of the separation zone form the protective layer which synthesizes defense-related proteins to protect the exposed surface from pathogen infection.

30.14 Mechanism of Abscission

The young leaves abscise only when they become old. Ethylene and auxin are known to play a key role in regulation of abscission. It is observed that if the lamina portion of the leaf is removed, the leaf soon abscises. This suggests that some factor produced in leaves moves from the blade to the petiole to prevent abscission. Experimental evidence suggests that this factor is IAA. If auxin is applied to the cut end of debladed petiole, abscission is prevented. At the same time, application of auxin is effective if applied at the beginning of the lag phase. Delayed application of auxin may have little or no effect. This indicates that auxin controls leaf abscission. Further, endogenous concentration of auxin in the leaves falls at the time of abscission (Fig. 30.16). Ethylene also plays a key role regulation of abscission. Whole plants exposed to ethylene gas show enhanced rate of leaf or flower abscission. Ethylene production is found to be sufficiently high in abscising organs. Ethylene-insensitive mutants in *Arabidopsis* show delayed senescence and abscission. Investigations have also shown that the relative concentration of auxin on two sides of the abscission layer regulate the production of ethylene that

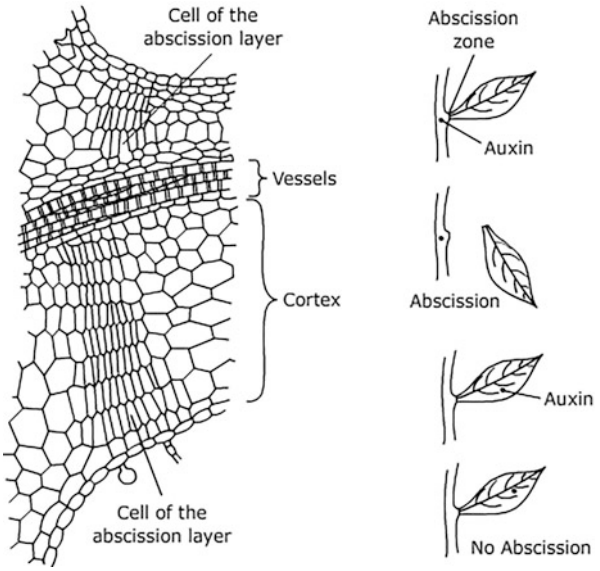


Fig. 30.16 Effect of auxin application on abscission

stimulates leaf abscission. The process of abscission is divided into three distinct phases during which there is increase in the sensitivity of the cells toward ethylene.

30.14.1 Leaf Maintenance Phase

At this stage there exists a gradient of auxin from the leaf blade to the stem, and the abscission zone remains in insensitive stage. There is no abscission.

30.14.2 Abscission Induction Phase

There is reduction in the auxin gradient in the leaf blade, and the abscission zone becomes sensitive to ethylene. Those treatments that enhance leaf senescence may do so by interfering with auxin gradient.

30.14.3 Abscission Phase

In this phase the cells of the abscission zone which are already sensitized respond to low concentration of endogenous ethylene. These cells synthesize and secrete wall-degrading enzymes resulting in cell separation and leaf abscission. This phase is marked by synthesis of new proteins. Inhibitors of transcription and translation (i.e., actinomycin D and cycloheximide, respectively) retard abscission if applied during early stage of development. They have little effect if applied at a later stage. Cell wall hydrolases (like endo-1,4-β-glucanases) and polygalacturonases are also synthesized upon induction of abscission by ethylene. In nature, senescence of an organ and its abscission usually go together. Cytokinins retard senescence, while ethylene promotes it. Also, ethylene promotes abscission, while auxin retards it. Young and mature leaves have abundant auxin which maintains a gradient in the abscission zone and prevents ethylene induction of abscission-related changes (abscission syndrome). Cytokinin in the leaf prevents induction of senescence in the leaf. As the leaves age, the endogenous concentration of both IAA and cytokinin drop, and this allows ethylene-induced senescence and abscission syndromes to be expressed.

30.15 Whole-Plant Senescence

To sum up, we need to understand if there is relationship between whole-plant senescence and organ senescence. We discussed in the beginning that the life span of plants varies from a few weeks to a few years (annuals, biennials, and perennials). Senescing tissues seem to be deficient in mechanisms that protect a plant against physiological decline. It is said that unlike annuals, perennials are better equipped to be able to survive through the deleterious effects of time (**mutational load**). Mutational load is the total genetic burden in a population resulting from

accumulated deleterious mutations. It is defined as a balance between selection against a deleterious gene and its production by mutation. It occurs when cell replication mechanisms propagate errors over thousands of years. Mutation rate can also increase further due to ROS accumulation with increasing age of the plant. Another difference between annuals and perennials lies in the determinate nature of their apical meristems. In annuals, all indeterminate vegetative shoot apices become determinate floral apices and the entire plant senesces after seed dispersal. In contrast, perennials retain a population of indeterminate shoot apices as well as those apices that become reproductive and determinate. Interestingly, ability to delay senescence by removal of reproductive structures is a characteristic feature of annuals. It is observed that repeated de-podding of soybean plants is an inducing factor for the plant to remain vegetative. Earliest explanation for this is that vital nutrients are redistributed from vegetative sources to reproductive sinks. Resource redistribution along with alterations in source-sink relationship caused by flower development may induce a shift in hormonal and nutrient balance of the plant leading to senescence.

Summary

- Senescence involves irreversible and degenerative changes in an organism, leading to death. These changes are self-digesting processes controlled by environment and the genetic makeup of an organism.
- Programmed cell death occurs via vacuolar-type PCD (involves vacuolar swelling and cell rupturing) and via hypersensitive response-type PCD (involves vacuolar water loss and cell shrinkage). Vesicles formed during senescence that engulf portions of cells to be degraded are called **autophagosomes**. There formation is regulated by autophagy-related genes and specific proteins. PCD remodels the plant at cellular, tissue, and organ level in important processes like tracheary element differentiation, lysigenous aerenchyma formation, trichome development, functional megaspore formation, etc.
- Leaf senescence involves nutrient relocation from leaves to other parts of the plant thereby exhibiting coordinated chain of events at cell, tissue, and organ level. Senescence process is regulated by several hormones where gibberellins, cytokinins, and brassinosteroids retard the process, while ABA, ethylene, and jasmonates enhance it. Senescence is affected by both external and internal factors through alteration of gene expression (NAC and WRKY being the most abundant gene families regulating the process). The process of senescence has also been associated with overproduction of reactive oxygen species (ROS).
- Senescence is followed by abscission, which is shedding of leaves and other plant organs through formation of abscission zone.

Multiple-Choice Questions

1. Catabolic process which is due to trauma caused by external agents and results in rupture of membranes and tissue inflammation:
   (a) Autophagy
   (b) Necrosis
   (c) Apoptosis
   (d) Degradation
2. A highly regulated energy-dependent process leading to programmed cell death in animals:
   (a) Necrosis
   (b) Autophagy
   (c) Apoptosis
   (d) Senescence
3. Which of the following cell organelle is degraded first during leaf senescence?
   (a) Peroxisomes
   (b) Mitochondria
   (c) Chloroplasts
   (d) Nuclei
4. The mechanism used by plants to prevent spread of infection by killing the cells around infection site to prevent its further spread is called:
   (a) Hypersensitive response
   (b) Vacuolar response
   (c) Autophagy
   (d) Camouflage

Answers

1. b 2. c 3. c 4. a

Suggested Further Readings

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates Inc, Sunderland, pp 665–692

Thomas H, Ougham H, Mur L, Jansson S (2015) Senescence and cell death. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. Wiley-Blackwell, Chichester, pp 925–982