International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Mechanisms and Regulation of Senescence: An Overview on Hormonal and Oxidative Perspective

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**Abstract:**
Senescence in plants represents a crucial developmental phase involving the regulated degradation of cellular components, enabling the remobilization of nutrients to support growth and reproduction. This review focuses on cotyledon senescence occurring during early seedling development. Cotyledon senescence is characterized by complex physiological, biochemical, and molecular processes including chlorophyll degradation, macromolecular breakdown, and dynamic hormonal signaling. Hormones such as ethylene, cytokinins, and abscisic acid (ABA) play antagonistic roles in regulating the timing and progression of senescence. Additionally, the interplay between reactive oxygen species (ROS) and antioxidant defense systems forms a pivotal aspect of senescence regulation, acting both as cellular damage agents and signaling molecules. Despite significant advances, the signaling networks linking embryonic axis development with cotyledon senescence continues to be lacking. Unraveling these pathways may offer insights into seedling establishment and improve our knowledge of plant developmental biology.

**Keywords:** Cotyledon senescence, Reactive oxygen species (ROS), Chlorophyll Biosynthetic Pathway, Cytokinin, Ethylene, Abscisic acid (ABA), Nutrient remobilization, Oxidative stress, Antioxidant defense.

## Introduction:

Programmed cell death (PCD) is crucial for the growth and development of eukaryotic organisms, including plants and animals. It is a genetically regulated process that allows for the self-destruction of cells (Lam, 2004). In plants, PCD occurs during various developmental stages and in response to environmental factors, primarily manifesting as senescence and the hypersensitive response (HR) (Greenberg, 1996; Mittler et al., 1996; Pennell and Lamb, 1997; Mc-Cabe and Leaver, 2000).

Senescence involves gradual tissue changes leading to the death of the organism or parts of it, evident throughout a plant's lifecycle (Woolhouse, 1978). While related, senescence and ageing are distinct; ageing, as defined by Medawar (1957), refers to time-related changes that can occur independently of death. Despite the distinctions, the relationship and differences among PCD, senescence, and ageing remain a complex area of study that is not yet fully elucidated.

Senescence is the final developmental phase of plant organs, essential for the plant lifecycle. It involves the selective disposal of unwanted cells after nutrient absorption, influenced by environmental and

internal factors. This highly regulated process includes the degradation of proteins, nucleic acids, and lipids, despite its catabolic nature being crucial for reproductive success by remobilizing nutrients to developing organs. Senescence affects various plant parts, highlighting the complexity of plant development and the balance between growth and nutrient allocation. The phenomenon of senescence is observed throughout the life cycle of plants, influencing different organs such as leaf senescence, cotyledon senescence, flower senescence, and fruit senescence (Fig. 1).

Cotyledon senescence is vital for plant development during germination, which occurs in two forms: hypogeal, where cotyledons stay underground, and epigeal, where they rise above the soil with the shoot. After germination, cotyledons may photosynthesize, but as they senesce, their photosynthetic activity declines due to the breakdown of chlorophyll and other cellular components. This process is affected by various internal and external factors, reflecting the complexity of plant development regulation.

The arrangement of senescence phenomena in the life cycle of seed plants:

**Fig. 1. Most of the phenomena listed, except those which occur within the seed, are integral parts of the developmental program of the plant.**

**Factors involved in the regulation of senescence:**

During different developmental stages of plants, senescence is regulated by many factors:

- External factors:

a) Nutrients
b) Temperature
c) Drought
d) Pathogen
e) Shade
f) Ultra violet light and ozone damage

- Internal factors:

a) Hormones (Abscisic acid, Auxin, Cytokinin, Ethylene, Jasmonic acid, Salicylic acid)
b) Reproductive factors

**Metabolic regulation in senescence:**

The study of senescence examines the aging process, focusing on its hormonal, molecular, and genetic regulation. In plants, cotyledon senescence marks the final maturation stage, involving the decay of

cotyledons and nutrient mobilization for seedling growth. This process, similar to leaf senescence, is driven by gene expression, metabolic changes, and cell structure alterations, particularly the breakdown of chloroplasts. As cotyledons cease normal functions, new metabolic pathways emerge, leading to the catabolism of proteins, chlorophyll, and lipids, and the hydrolysis of storage compounds to supply nutrients for seedlings.

**(i) Chlorophyll degradation**

Leaf senescence is marked by chlorophyll loss, causing a shift from green to yellow. However, chlorophyll degradation does not consistently align with the leaf's photosynthetic ability, as some mutants can senesce without losing chlorophyll (Woolhouse, 1967; Hardwick et al., 1968). This suggests that chlorophyll breakdown may not be directly tied to senescence. During senescence, chloroplasts transform into gerontoplasts, losing thylakoid membranes and accumulating plastoglobuli (Krupinska, 2006). Electron microscopy has shown chloroplasts in the central vacuole, which contains lytic hydrolases (Wittenbach et al., 1982; Minamikawa et al., 2001), but there is no direct evidence of chloroplast movement into the vacuole in living cells, and the transport mechanisms remain unclear (Hörtensteiner and Feller, 2002; Krupinska, 2006).

Senescence is characterized by the degradation of chlorophyll, a crucial pigment for photosynthesis that stabilizes light-harvesting complex proteins (Kuttkat et al., 1997). Disruptions in chlorophyll biosynthesis lead to diminished photosynthetic activity and can result in the accumulation of tetrapyrrole intermediates. These intermediates, when exposed to light, can generate reactive oxygen species (ROS) and also act as signals to regulate the expression of nuclear genes related to both photosynthetic and non-photosynthetic proteins (Gupta and Tripathy, 2000; Vavilin and Vermaas, 2002). Consequently, any perturbation in chlorophyll biosynthesis can negatively impact plant growth. The biosynthesis of chlorophyll begins with the synthesis of 5-aminolevulinic acid (ALA) from glutamic acid through three enzymatic steps (Fig. 2). Eight ALA molecules are then converted into protoporphyrinogen IX, which is oxidized to protoporphyrin IX (Proto IX) by protoporphyrinogen IX oxidase. This process branches into two pathways: one leading to the formation of Mg-protoporphyrin IX (Mg-proto IX) through Mg-chelatase, and the other leading to protoheme via Fe-chelatase. Mg-proto IX is further modified to produce Mg-protoporphyrin IX monomethyl ester (Mg-proto IX ME), which is then converted to divinyl protochlorophyllide a (DVPchlide) and subsequently to monovinyl protochlorophyllide a (MVPchlide). Upon illumination, MVPchlide is reduced to chlorophyllide a (Chlide a), which is then esterified to form chlorophyll. This intricate pathway highlights the complexity of chlorophyll biosynthesis and its critical role in plant health and development.

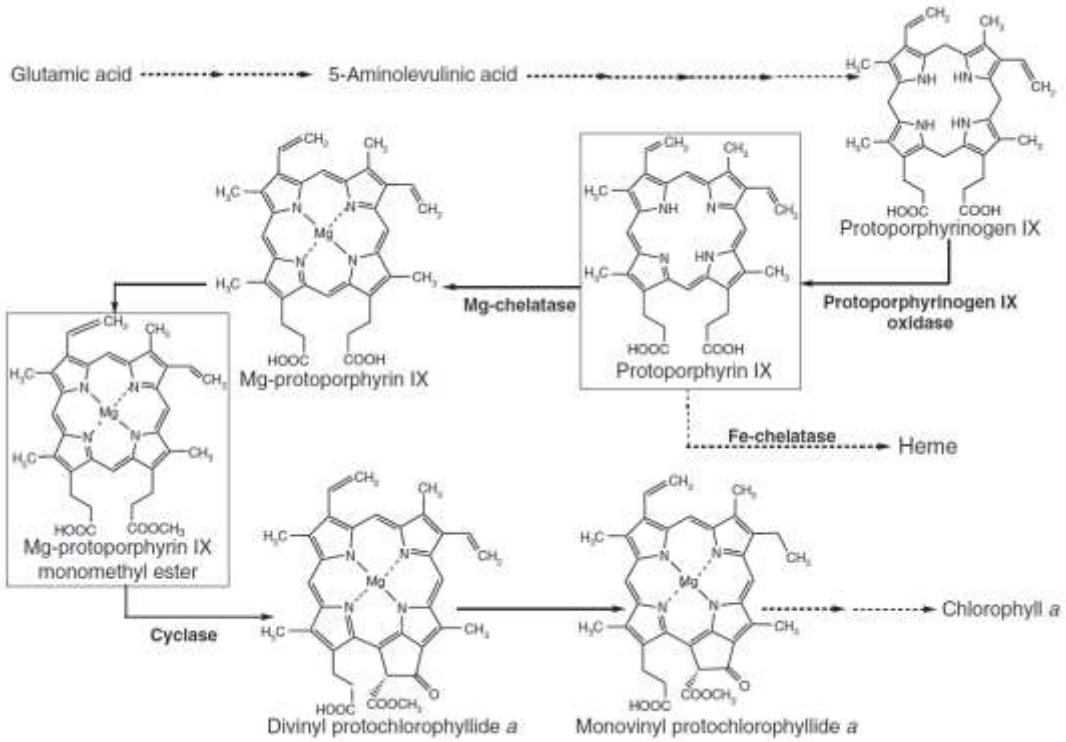


**Fig. 2. Structural outline of chlorophyll biosynthetic pathway (Aarti et al., 2006)**

A chlorophyll degradation pathway (Fig. 3) initiates with the dephytylation of chlorophyll, a reaction catalyzed by chlorophyllase (Chlase), resulting in the formation of phytol and chlorophyllide (Takamiya et al., 2000; Harpaz-Saad et al., 2007). Following this, the central magnesium ion ($Mg^{2+}$) is removed from chlorophyllide by Mg-dechelatase, leading to the cleavage of the porphyrin macrocycle of pheophorbide. This cleavage is facilitated by the combined actions of pheophorbide a oxygenase (PAO) and red Chl catabolite (RCC) reductase (RCCR) (Hörtensteiner et al., 1995; Rodoni et al., 1997). The outcome of this reaction is a primary fluorescent catabolite (FCC), which is subsequently transformed into non-fluorescent chlorophyll catabolites (NCCs) (Oberhuber et al., 2003). These biochemical transformations are collectively referred to as the "PAO/phyllobilin" pathways, highlighting the pivotal role of PAO enzymes in the loss of the green pigment characteristic of chlorophyll (Hörtensteiner, 2006; Hörtensteiner et al., 2019).

Pathways for chlorophyll degradation are same in case of both fruit ripening and leaf senescence (Hörtensteiner and Kräutler, 2011). Chlorophyll biosynthesis in cotyledons is essential for plant growth as it boosts photosynthetic activity. This process is vital for cotyledon development, after which senescence begins, characterized by chlorophyll degradation that reduces photosynthesis. Once chlorophyll synthesis peaks, degradation starts, marking the onset of senescence, which progresses alongside chlorophyll loss, illustrating the interconnection of these developmental processes.

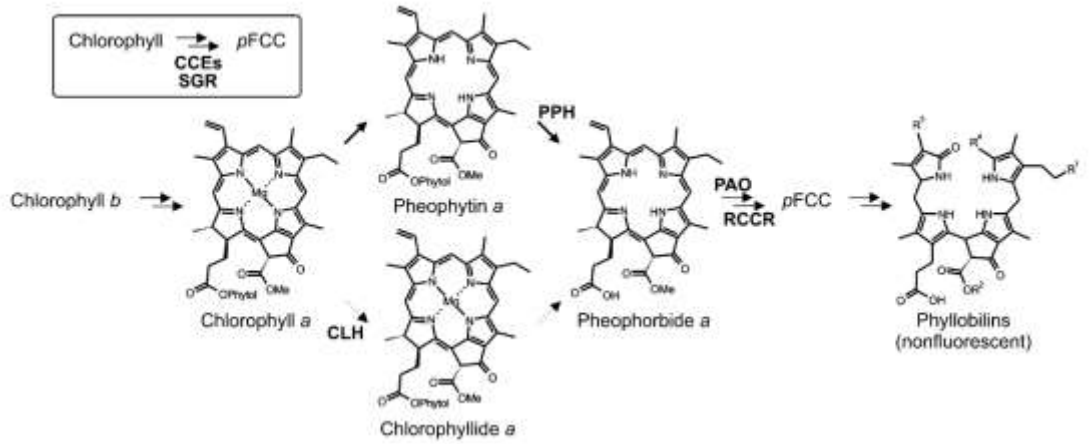


**Fig. 3. Structural outline of chlorophyll degradation pathway (PAO/phyllobilin pathway; Guyer et al., 2014). $R^1$ to $R^4$ indicate sites of modifications that are found in nonfluorescent phyllobilins of different plant species (Kräutler and Hörtensteiner, 2013).**

**(ii) Degradation of macromolecules**

Macromolecules such as proteins, sugars, lipids, and nucleic acids are integral components of cotyledon cells. During seed germination, these macromolecules undergo degradation into simpler forms, which are then mobilized through the plant's axis to support seedling growth. The senescence of cotyledons marks the final phase of germination, wherein the remaining macromolecules are utilized for the development of seedlings. This process of senescence is highly regulated and involves the deterioration of both macromolecules and cellular organelle structures.

In the programmed cell death (PCD) associated with senescing storage cotyledons, vacuoles transition from serving as protein storage organelles to becoming large central vacuoles. Cotyledons also contain lipids stored in specialized organelles called oleosomes, which are eliminated during senescence. The metabolism of lipids in these storage tissues is linked to the formation of glyoxysomes, organelles that play a crucial role in gluconeogenesis. Additionally, in senescing photosynthetic tissues, peroxisomes are transformed into glyoxysomes. These observations underscore that senescence is a programmed and systematic process within plant development.

Chloroplast degradation is a significant aspect of leaf senescence, with the majority of cellular proteins, particularly Rubisco and chlorophyll binding light harvesting proteins, localized within these organelles. Rubisco (EC 4.1.1.39), representing about 50% of the soluble protein in chloroplasts (Wittenbach, 1978), is quickly degraded in the early phases of senescence, though this degradation process tends to slow down in the later stages (Friedrich and Huffaker, 1980; Mae et al., 1984). Chloroplasts are believed to contribute organic nitrogen from senescing tissues, primarily through the degradation of key proteins such as Rubisco (EC 4.1.1.39) and chlorophyll-binding light-harvesting proteins. Despite its importance, the specific mechanisms behind the degradation of intrachloroplastic Rubisco remain unclear (Feller et al., 2008). Research has identified serine protease activity in senescing wheat leaves, indicating that Rubisco is a target for such enzymes, yet the full spectrum of enzymes involved in chloroplast protein degradation during senescence has not been elucidated (Roberts et al., 2003).

The loss of key cellular components significantly contributes to senescence. Historical insights indicate that plant proteins are dynamic, undergoing continuous breakdown and resynthesis, leading to the protein cycle theory. This theory posits that proteins are degraded into amino acids for immediate use or

for synthesizing new proteins, underscoring the complexity of protein metabolism and its importance in senescence.

Cellular senescence is linked to the loss of essential components. In the 19th century, the idea emerged that plant proteins are in a constant state of breakdown and resynthesis, leading to the protein cycle theory (Davies, 1980). This theory suggests that proteins are degraded into amino acids, which are then used for cellular functions or resynthesized into new proteins. This underscores the significance of protein turnover for cellular health.

Protein synthesis and degradation are crucial for the normal development, homeostasis, and eventual death of plant cells (Vierstra, 1996). The process of proteolysis in plants is intricate, involving a variety of enzymes and multiple proteolytic pathways that operate in different cellular compartments (Grudkowska and Zagdańska, 2004). Both ATP-dependent and ATP-independent pathways play significant roles in plant proteolysis (Callis, 1995). Specifically, chloroplast proteins can be degraded through several mechanisms, including vacuolar proteases, the ubiquitin pathway in the cytosol, and the plastidial Clp-system (Shanklin et al., 1995; Vierstra, 1996). These findings highlight the complexity and versatility of protein degradation processes in plant cells, underscoring their importance in cellular function and regulation. The death of cotyledon storage parenchyma cells initiates with the mobilization of reserves and the process of vacuolation. During the programmed cell death (PCD) dismantling phase, these cells undergo progressive autolysis, leading to the development of a large central vacuole. As senescence progresses, this vacuole expands and eventually ruptures, releasing hydrolytic enzymes that facilitate the degradation of cellular contents, as noted in the literature by Beers (1997) and van Doorn and Woltering (2005). In the final stages of PCD, the protoplast retracts from the cell wall, culminating in the complete lysis of the cytoplasm and nucleus, alongside the condensation of the protoplast away from the cell wall, (Reape et al., 2008). This sequence of events highlights the intricate processes involved in cellular degradation during plant senescence.

The degradation of internucleosomal DNA during apoptosis is linked to the activation of endogenous $Ca^{2+/}Mg^{2+}$ -dependent endonucleases, as highlighted in studies by Mittler and Lam (1995), Xu and Hanson (2000), and He and Kermode (2003b). In plants, the modulation of nucleases is observed both in developmental stages and in response to various internal and external signals (Sugiyama et al., 2000). Mitochondria are implicated in the initiation of programmed cell death (PCD) by releasing cytochrome c into the cytoplasm when exposed to death-promoting factors, including heat shock and certain chemical treatments (Balk et al., 1999; Stein and Hansen, 1999; Sun et al., 1999 and Vacca et al., 2006). This suggests that during senescence, which is a programmed process akin to PCD and apoptosis, endogenous mitochondrial cytochrome c and $Ca^{2+/}Mg^{2+}$ -dependent endonucleases may significantly contribute to the initiation of senescence and the degradation of internucleosomal DNA.

**Hormonal regulation in senescence:**

Plant hormones play a crucial role in regulating senescence, with ethylene, cytokinin, and abscisic acid (ABA) being particularly significant in influencing its initiation, timing, and rate. Research indicates that ABA and ethylene are associated with the acceleration of senescence symptoms, while cytokinin is known to delay the onset of this process (Smart, 1994; Badenoch-Jones et al., 1996). Although ethylene is not classified as an initiator of senescence, its production increases during the senescence phase (Orzáez et al., 1999). Furthermore, both endogenous (Van Staden et al., 1988) and exogenously (Gan

and Amasino 1995, Jordi et al., 2000, McCabe et al., 2001) applied cytokinin have been shown to effectively postpone senescence, highlighting its potential as a regulatory agent in plant aging processes.
Ethylene is identified as the most potent promoter of senescence in plants, influencing the aging process of leaves, flowers, and the ripening of fruits. The production of endogenous ethylene is closely linked to senescence; for instance, leaves subjected to dark treatment exhibit increased ethylene production as they undergo senescence. The role of ethylene in regulating senescence is particularly evident in the context of fruit ripening, where two key enzymes, ACC synthase and ACC oxidase, facilitate the final stages of ethylene biosynthesis. Inhibitors of ethylene biosynthesis and action have been shown to effectively impede both fruit ripening and leaf senescence. Notable inhibitors include 1-aminoethoxyvinylglycine (AVG) and amino-oxyacetic acid (AOA), which target ethylene biosynthesis, while silver thiosulfate (STS), silver nitrate, and 1-methylcyclopropene (1-MCP) inhibit ethylene's action. It is important to note that ethylene does not function in isolation; its effectiveness in inducing senescence is contingent upon the developmental stage of the plant organs. For example, ethylene can induce senescence in cotyledons, indicating that these organs must be responsive to ethylene biosynthesis or action inhibitors for the process to occur.
Cytokinins (CKs) are crucial plant hormones known for their role in regulating cell division, but they also influence a variety of physiological processes. These include seed development, inhibition of root growth, branching, and enhancing resistance to environmental stresses (Richmond and Lang, 1957; Mok, 1994). CKs produced in the roots, plays a crucial role in regulating leaf senescence by being transported to the leaves. Its function is notably opposite to that of ethylene, as cytokinin inhibits or delays the senescence process. Evidence supporting the role of CK in blocking senescence includes the observation that endogenous CK concentrations decrease in most senescing tissues, and the application of exogenous CKs can effectively delay senescence across various tissues. Furthermore, CK influences the transcription of senescence-associated genes, either directly or through a signaling pathway, thereby contributing to the regulation of the senescence process in plants. Additionally, CKs contribute to delaying senescence, which is the aging process in plants. Research indicates that CKs are significantly involved in the greening process (Mikulovich et al., 1971; Mlodzianowski and Gesela, 1974; Longo et al., 1990; Smart et al., 1991; Smigocki, 1991) and the development of plastids, such as etioplasts and chloroplasts (Khokhlova et al., 1971; Parthier, 1979). They also play a role in the activity of several plastidial enzymes (Feierabend, 1969; Romanko et al., 1976; Feierabend and de Boer, 1978; Khokhlova et al., 1978). Notably, a decline in endogenous CK levels has been linked to the onset of senescence, as evidenced by studies on cotyledon senescence in Cucurbita and natural leaf senescence in maize (He et al., 2005). This suggests that maintaining CK levels may be vital for prolonging plant vitality and delaying aging processes.
Abscisic acid (ABA) is a sesquiterpenoid synthesized from xanthophylls, playing a crucial role in various physiological processes (Taylor et al., 2000). It regulates seed maturation and dormancy, responds to environmental stresses, influences shoot elongation, and affects the morphogenesis of submerged plants (Kuwabara et al., 2003; Sharp and LeNoble, 2002). Additionally, ABA is vital for maintaining root growth and facilitating organ abscission. In terms of its impact on senescence, ABA has been shown to accelerate the senescence process in isolated leaves across different species, particularly at high concentrations ranging from 50 to 100 mg/l in attached leaves (El-Antably et al., 1967). In detached tobacco leaves, the early stages of senescence are marked by an increase in ABA levels, which subsequently decline (Even-Chen and Itai, 1975). This indicates a complex relationship

between ABA concentration and the senescence process, highlighting its significant role in plant development and stress responses.

**Senescence associated genes:**

Senescence associated genes (SAGs) exhibit diverse nomenclature across different species, with notable examples including LSC from Brassica napus, SAG from Arabidopsis, and SENU from tomato. Approximately 50 cDNAs have been identified that promote senescence functions based on sequence homology, with a subset validated through biochemical and physiological studies. SAGs can be categorized into three types of cysteine proteases: the first type encompasses enzymes that are markedly induced during the germination of cereal seeds; the second type is akin to papain-like enzymes; and the third type includes enzymes that facilitate protein processing.

The expression patterns of SAGs are variable; some are activated early in the senescence process while others are expressed later. Additionally, the expression can occur in both attached and detached tissues, and certain genes are specific to particular organs. Notably, SAGs involved in gluconeogenesis are expressed in senescing leaves, cotyledons, and germinating seeds, although the characterization of SAGs in cotyledon senescence remains limited.

**Senescence and ROS:**

Radicals are defined as atoms, molecules, or ions that possess unpaired electrons in an open cell configuration, which renders them highly reactive in chemical processes. Reactive oxygen species (ROS) are a specific category of these highly reactive molecules, characterized by the presence of oxygen ions and peroxides, and is known to induce various forms of cellular damage. During the process of senescence, there is an increased production of ROS, including superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), hydrogen peroxide ($H_2O_2$), and singlet oxygen ($^1O_2$). Additionally, these reactive species are byproducts of normal enzymatic reactions occurring in cellular organelles such as peroxisomes, glyoxysomes, and chloroplasts. Understanding the role of radicals and ROS is crucial for comprehending their impact on cellular health and aging processes.

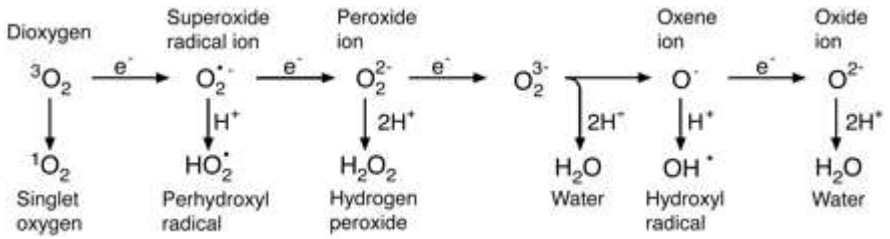


**Fig. 4. The production of various reactive oxygen species (ROS) through energy transfer or the sequential univalent reduction of ground-state triplet oxygen**

Reactive oxygen species (ROS) are highly reactive molecules with varying degrees of stability. Among these, hydroxyl radicals ($OH^{\cdot}$) are the least stable yet most damaging, while hydrogen peroxide ($H_2O_2$) is more stable and less toxic. The instability of $OH^{\cdot}$ limits its mobility, confining its reactions to the site of production, whereas $H_2O_2$ can diffuse to other cellular compartments, functioning as a signaling molecule.The primary targets of ROS include cellular proteins, membrane lipids, and macromolecules such as chlorophylls. The oxidative damage caused by free radicals can trigger chain reactions,

particularly evident in lipid oxidation, leading to the generation of additional ROS. Furthermore, ROS can interconvert through enzymatic actions or spontaneous processes, highlighting their dynamic nature within biological systems.

The free radical theory of ageing highlights the significant role of reactive oxygen species (ROS) in the ageing process, as proposed by Harman and Piette in 1966 and further developed by Harman in 1972. In plants, ROS are primarily generated through photochemical reactions, alongside other free radicals produced via various enzymatic and non-enzymatic processes, which are integral to plant cell metabolism. To mitigate the damaging effects of ROS, cells employ a defense mechanism that includes a variety of antioxidants. Key enzymes such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) play crucial roles in scavenging free radicals. Additionally, non-enzymatic antioxidants like ascorbate, reduced glutathione (GSH), α-tocopherol, and carotenoids contribute to this protective strategy (Foyer et al., 1994; Bartoli et al., 1996; Hodges et al., 1996, 1997a, 1997b). Among these, the ascorbate-glutathione cycle, also referred to as the Asada-Halliwell cycle, is recognized as the most vital antioxidant system during cellular senescence. The presence of multiple sources of ROS in senescing cells underscores the complexity of oxidative stress management in plant biology.

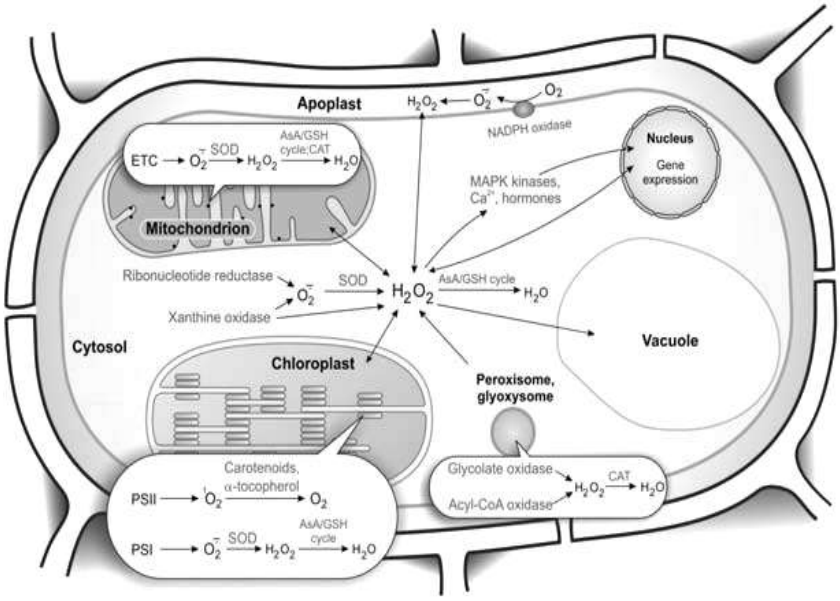


Fig. 5. The picture discusses the production and metabolic fate of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), superoxide anion radical ($O_2^{\cdot-}$), and singlet oxygen ($^1O_2$) within various cellular compartments. It highlights the involvement of major enzymes and nonenzymatic components in maintaining ROS homeostasis. Plants are noted for their diverse sources of ROS and their sophisticated mechanisms for scavenging and utilizing these reactive species. Abbreviations are as follows: $H_2O_2$, hydrogen peroxide; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, superoxide radical. PSI, Photosystem I; PSII, Photosystem II; ETC, Electron transport chain; SOD, superoxide dismutase; CAT, catalase; AsA/GSH, ascorbic acid/glutathione. (Gadjev et al., 2008).

Reactive oxygen species (ROS) are primarily produced in plants through photochemical reactions in chloroplasts and various enzymatic and non-enzymatic processes, serving as essential reactants for cellular metabolism. ROS significantly impact hormonal signaling pathways, with evidence supporting their integration into hormone signaling processes (Wilhelmová et al., 2004; Mori et al., 2009).

Specifically, superoxide ($O_2^{\cdot -}$) is generated in the extracellular space by NAD(P)H oxidase (NOX) and is correlated with increased ethylene production. $O_2^{\cdot -}$ can be converted to hydrogen peroxide ($H_2O_2$), which functions as a cellular signal and is involved in cell wall remodeling. The conversion of $O_2^{\cdot -}$ to $H_2O_2$ can occur spontaneously or be catalyzed by superoxide dismutase (SOD) (Mori et al., 2009).

$H_2O_2$ plays a dual role in plants; it is involved in cross-linking cell wall components and acts as a local trigger for programmed cell death (PCD), initiating protective gene expression in adjacent cells (Levine et al., 1994). Its accumulation in the apoplastic space is induced by abscisic acid (ABA) (Hu et al., 2005, 2006). SODs, which are crucial for converting $O_2^{\cdot -}$ to $H_2O_2$, are classified into three groups (Mn SOD, Cu/Zn SODs and Fe SOD) based on their metal cofactor requirements. $H_2O_2$ can be scavenged by various enzymes, including catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), as well as through cycles like the ascorbate-glutathione cycle and/or the glutathione peroxidase (GPX) cycle.

Like catalase, POX and APX also act towards scavenging $H_2O_2$. POX not only reduces $H_2O_2$ but can also generate ROS (e.g. •OH, HOO•) and facilitate the polymerization of cell wall materials. Class III plant peroxidases (Welinder, 1992) play a crucial role in the reduction of $H_2O_2$ by utilizing electrons from donor molecules, such as phenolic compounds, lignin precursors, auxin, or secondary metabolites (Hiraga et al., 2001). Additionally, POX is implicated in chlorophyll degradation, occurring in both chloroplasts and vacuoles (Yamauchi et al., 2004). Conversely, ascorbate serves as a particular electron donor for APX, facilitating the reduction of $H_2O_2$ to water and leading to the formation of monodehydroascorbate (Shigeoka et al., 2002). In a similar manner, GPXs utilize GSH or other comparable substrates to reduce $H_2O_2$, along with organic hydroperoxides and lipid peroxides (Mittler et al., 2004). A decline in SOD activity is indicative of the onset of senescence, as it leads to increased ROS accumulation (Srivalli and Khanna-Chopra, 2001). Similar declines in catalase and APX activities have been observed during the senescence of various plant leaves, highlighting the critical role of antioxidant enzymes in managing ROS levels and plant health (Pastori and del Río, 1994; Jimenéz et al., 1998; Procházková et al., 2001).

Genetically controlled cell death, or programmed cell death (PCD), can be initiated by various reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), and superoxide ($O_2^{\cdot -}$). The process involves multiple components, including proteins that participate in the early signaling stages, such as kinases and phosphatases, which help relay and amplify the ROS signal. Additionally, transcription factors play a crucial role in orchestrating the global transcriptional changes that lead to the execution of cell death. Environmental and developmental signals can enhance ROS production either directly or through the activation of ROS-inducing genes, including NADPH oxidases and peroxidases. This increase in ROS can lead to the upregulation of ROS-inducing genes while downregulating ROS-scavenging genes, thereby amplifying the ROS signal. Conversely, ROS can also promote the expression of scavenging genes, which inhibit ROS production, resulting in a reduction of ROS levels back to baseline. The initial signaling components involved in PCD are often linked to the generation of ROS, while others are tasked with modulating ROS levels through antioxidant mechanisms, such as catalases and ascorbate peroxidases (APXs) (Gadjev et al., 2008).

The phenomenon of senescence is correlated with PCD, suggesting that signals from one organ may influence another, potentially involving ROS as signaling molecules. For instance, during germination, signals from the growing embryonic axes may induce senescence in cotyledons. Hormones like ethylene and abscisic acid (ABA) are likely to act upstream in this signaling cascade, with existing literature

indicating interactions between hormones and ROS. These hormones may utilize ROS as downstream effectors to regulate gene expression through redox-sensitive proteins and transcription factors.

Reactive oxygen species (ROS) play a dual role in plants, acting both as agents of cellular damage and as crucial signals in defense responses. At low concentrations, ROS can trigger the induction of defense mechanisms and adaptive responses, while at high concentrations, they can lead to significant cellular damage and cell death. This highlights the importance of maintaining ROS homeostasis through precise regulatory mechanisms at the cellular level. Despite extensive research on the sources of ROS and the systems that scavenge oxidants, the exact mechanisms governing the temporal and spatial regulation of ROS homeostasis remain unclear. Additionally, there is potential for crosstalk between ROS and other signaling pathways, suggesting a complex interplay in plant signaling networks. Recent studies are increasingly focusing on the role of ROS as intrinsic signals in plant growth and development, underscoring their significance in plant biology.

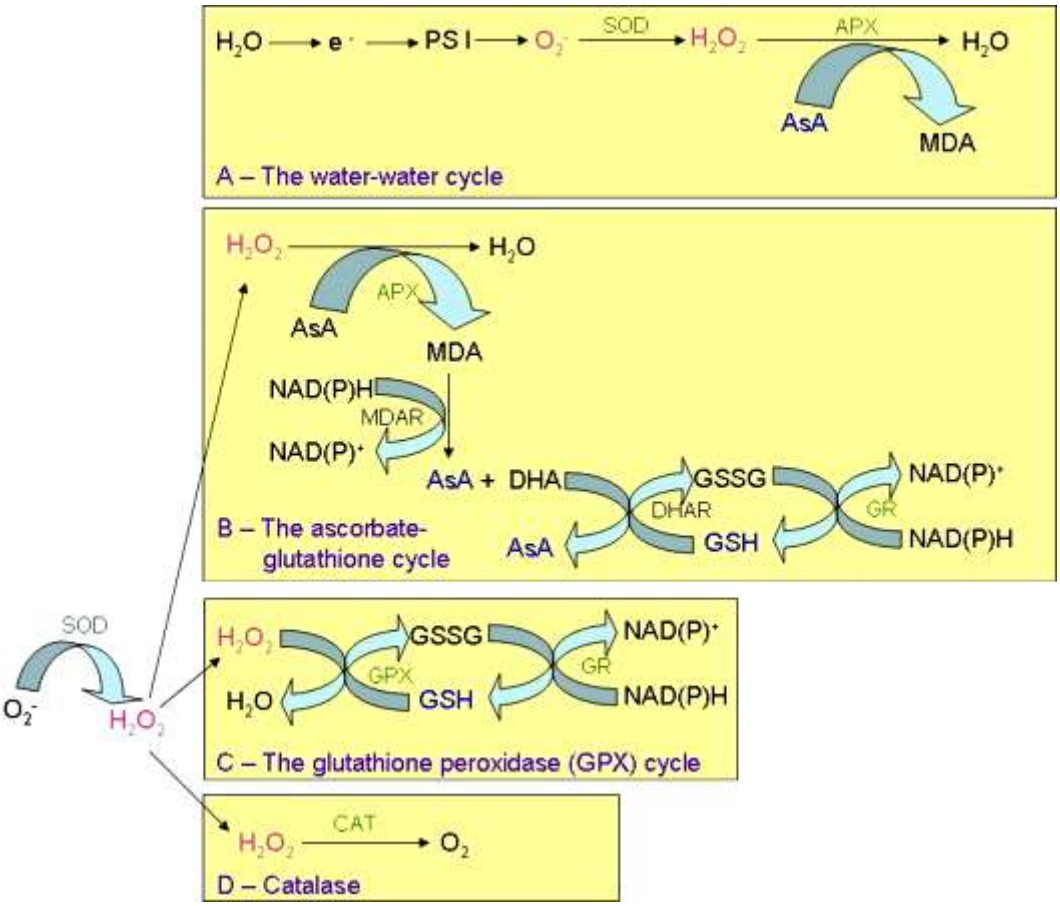


**Fig. 8.** The enzymatic ROS scavenging by superoxide dismutase (SOD), catalase (CAT), the ascorbate glutathione cycle, the glutathione peroxidase (GPX) cycle. SOD converts the superoxide to hydrogen peroxide. CAT converts the hydrogen peroxide to oxygen and water. $H_2O_2$ also converted into water by ascorbate-glutathione cycle. [APX-ascorbate peroxidases; MDA-monodehydroascorbate; DHA-dehydroascorbate; DHAR-DHA reductase; GR-glutathione reductase]

**Conclusion:**

Senescence represents the final developmental phase of an organ or the entire plant, characterized by overall deterioration driven by hydrolytic enzymes. The degradation patterns and signaling mechanisms

that initiate senescence differ significantly across various organs and plant species, influenced by their growth habits and habitats. Cotyledons serve as storage organs that support the developing plant until it becomes nutritionally independent through photosynthesis. Once this independence is achieved, cotyledons are programmed to die, although the duration of this phase varies among species, particularly during early developmental stages.

The embryonic axis, which develops into the plant body, plays a crucial role in regulating the metabolism of cotyledons, ultimately leading to their termination. The early developmental changes in the growing axes, particularly the establishment of photosynthetic systems that enable autotrophy, must be linked to the degradative processes occurring in cotyledons. This relationship is likely mediated by a signaling system that connects these two processes. Investigating this signaling system and its molecular components presents a significant challenge, yet it is essential for understanding seedling establishment, which is critical for plant success.

Being the last phase of development for an organ or the whole plant senescence comprises of over all deterioration by the concerted action of batteries of hydrolytic enzymes. But the pattern of degradation and above all the signaling involved to trigger such process is expected to vary definitely from organ to organ and plant to plant depending on the habit and habitat. Cotyledons are storage organs support the growing axes unless the latter become nutritionally independent i.e. photosynthetically competent. Once this is served cotyledons are destined to die, but the duration of this phase is different for plant species differing in early developmental stages. Thus, it appears that the embryonic axis that gives rise to the plant body has a definite impact on the metabolism of cotyledons leading to the termination of these organs. Early changes in the growing axes associated with the development of photosynthetic systems to make it autotrophic must be correlated with the degradative changes in cotyledons. Such correlation must be supported by some signaling system that links these two systems. It would be a challenging job to explore such signaling system including its components at the molecular level that would result in a knowledge-based command over the process of seedling establishment which is damn need for success of a plant.

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