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Nitric Oxide Signaling During Senescence and Programmed Cell Death in Leaves

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1. Introduction

Senescence is a ubiquitous developmental process that leads to the death of a cell, an organ, or an organism and occurs at the final stage of their development. There is a striking divergence and convergence between plants and animals regarding senescence regulation (Kenyon, 2001). The mechanisms of regulation of ageing in animals including p53, telomerase and telomere dynamics, DNA damage sensing and repair, and transcriptional activation and inactivation by histone acetylation/deacetylation are either not present or do not appear to play an important role in plant ageing. On the contrary, plants have evolved their own unique senescence-regulating mechanisms. These include the modulation of senescence by phytohormones, photosynthetic machinery, and protein degradation. In plants, the chloroplast is reported to be the first origin and target for initiating senescence (Misra and Biswal, 1980, 1981; 1982a, b, c, Biswal et al., 2001; Dilnawaz et al. 2001; Misra et al. 2011a), whereas in animals the mitochondrion serves as the initiator (Thomas, 2002). Thus, we may infer that plants and animals have evolved conserved strategies for the regulation of senescence, while employing diverse molecular mechanisms that have been shaped during the long history of evolution. Senescence is a genetically-controlled developmental programme, but it has no adaptive advantages in animals, except that in plants leaf senescence is a recruited nutrient recycle programme and hence is considered to have a strong adaptive advantage (Bleecker, 1998). This is the final developmental phase of a leaf which starts with nutrient salvage and ends with cell death. However, until late in senescence the process requires cell viability and is often reversible (Thomas *et al*., 2003). There has been some debate about the degree of overlap of senescence and PCD (Thomas *et al*., 2003; van Doorn & Woltering, 2004, 2008). van Doorn & Woltering (2004, 2008) identified three positions regarding the overlap in senescence and PCD. Some authors assumed total overlap (Noodén, 2004), but van Doorn & Woltering (2004, 2008) postulated that overlap is complete and senescence and PCD are synchronous. However a minority, argued that there was no overlap (Delorme *et al*., 2000; Thomas *et al*., 2003). van Doorn & Woltering (2008)

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defined PCD as 'the process that leads to the moment of death and the degradation that goes on after this moment'. Senescent cells are being actively recycled, which means that the cells are subjected to nucleases, proteases and photosynthetic breakdown along with various other senescent-related stresses. Normally one might expect that a cell which is subjected to this type of stress would activate PCD.

2. Symptoms associated with leaf senescence

Chloroplasts are the first organelles in leaves to show the symptoms of leaf senescence (Misra and Biswal, 1980, Dilnawaz et al., 2001; Misra et al., 2002; Misra et al. 2011a). Chlorophyll (Chl) is the key photosynthetic pigment required for the absorption of sunlight and photo-energy transduction by plants for primary productivity on earth. Absorbed energy can also be transferred from Chl to oxygen, resulting in the production of reactive oxygen species (ROS) (Apel and Hirt,2004). Likewise, inhibition of Chl biosynthesis or degradation can lead to the accumulation of phototoxic intermediates and ROS production (Apel and Hirt,2004; Pruzinska et al.2007).

There is a stepwise degradation of Chl (Hortensteiner 2006) during leaf senescence or accelerated cell death caused by various biotic or abiotic stresses (Matile et al., 1999). Hence, leaf yellowing is regarded as an external symptom of programmed cell death processes in senescing leaf cells (Nooden et al., 1997; Hortensteiner and Matile, 2004). The first visible event during senescence is leaf yellowing (Misra and Biswal, 1980; Quirino et al., 2000). Photosynthesis gradually slows down (Dilnawaz et al., 2001; Misra et al., 2002; 2010; 2011a; Misra and Misra, 1987; 2010; Misra and Biswal, 1982) and finally stops, but mitochondria and nucleus remain functional and respiration continues until the end of the senescence (Srivastava, 2002). Besides leaf age, darkness is more commonly considered to be an inducer of leaf senescence and induces loss of chlorophyll (Misra and Biswal, 1980, 1981), nucleic acid and protein (Misra and Biswal, 1982b), increase in the photosynthetic excitation pressure (Misra et al., 2011a) and alterations in the thylakoid membrane function such as photosystem I (PS I) and PS II activities (Biswal et al., 2001; Misra and Biswal, 1982a; Misra, 1993a). The expression of various photosynthesis associated genes was reduced (Misra, 1993b; Kleber-Janke and Krupinska, 1997; Weaver et al., 1998).

A decrease in nucleic acids occurs during leaf senescence (Misra and Biswal, 1982b). Total RNA levels are rapidly reduced along with the progression of senescence (Misra, 1993b; Taylor et al., 1993). Their initial decline is apparent in the chloroplast rRNAs and cytoplasmic rRNAs. This decrease in rRNAs contents is followed by that of the cytoplasmic mRNA and tRNA, and is accompanied by enhanced activity of several RNases. The earliest structural evidence for senescence appears in the chloroplast, manifested as changes in the grana and the formation of lipid droplets. Polysomes and ribosomes generally decrease fairly early, reflecting diminished protein synthesis. In comparison, the mitochondria and nucleus, both essential to energy production and gene expression, remain intact until the last stages (Quirino et al., 2000). This is because the senescing cells must be functional for progression of senescence until a late stage of senescence, possibly for the efficient re-utilization of cellular materials. In the final stage of senescence, when the leaves have turned almost completely yellow, typical symptoms of PCD, such as controlled vacuolar collapse, chromatin condensation, and DNA laddering have been reliably detected in naturally senescing leaves from a variety of plants including rice, tobacco, and five trees (Buchanan-

Wollaston et al. 2003). Eventually, disintegration of the plasma and vacuolar membranes become apparent, and the loss of integrity in the plasma membrane then leads to a disruption in cellular homeostasis, ending the life of a cell (Buchanan-Wollaston et al. 2003). Lipid-degrading enzymes, such as phospholipase D, phosphatidic acid phosphatase, lytic acyl hydrolase, and lipooxygenase, appear to be involved in the hydrolysis and metabolism of membrane lipids (Thompson et al., 2000). The majority of fatty acids is either oxidized to provide energy for the senescence process or processed to α-ketoglutarate via the glyoxylate cycle. This α-ketoglutarate can be converted into phloem-mobile sugars through gluconeogenesis or else used to mobilize the amino acids released during leaf protein degradation (Buchanan-Wollaston, 1997).

3. Factors regulating senescence in leaves

Leaf senescence is controlled by various internal and external factors including leaf age, light conditions, nutrient supply and environmental stress (Lim and Nam 2005). Plants integrate these factors through endogenous signaling molecules and coordinate the senescence process. Senescence is under genetic control and requires differential expression of specific genes. Expression of photosynthesis-associated genes (*PAGs*) is downregulated, while many other genes, designated as senescence-associated genes (*SAGs*), are upregulated during senescence (Lim and Nam, 2005; Lim et al., 2003).

During plant growth light dosage has an effect on ageing; high light intensity results in premature senescence when compared with growth under standard light intensities, while low light intensities delay the senescence process (Misra 1995; Misra and Misra, 1987; 1989a, b; 1995; Nooden et al., 1996).

Many other stress-inducing conditions such as drought, darkness, high temperature, high light, ozone, and pathogen attack can hasten leaf senescence as well (Biswal et al., 2001; Misra et al., 2001a, b; Misra and Misra 1986a, b; 2002; Misra and Biswal 1980, 1981, 1982a, b, c; 1987; 2000; Lim and Nam, 2005).

3.1 Hormonal regulation of leaf senescence

Plant hormones play an important roles in the regulation of the onset of senescence (Misra and Biswal 1980; Misra and Misra 1989a; 1991). All the identified phytohormones are reported to be involved in leaf senescence. Among the five classic hormones, the roles of ethylene and cytokinin in leaf senescence have long been established. Besides, jasmonic acid, salicylic acid, nitric oxide, and brassinosteroid are also implicated in regulating leaf senescence.

Cytokinins play a master regulatory role in leaf senescence (Misra and Biswal, 1980). While increasing cytokinin production could delay leaf senescence (Gan and Amasino, 1995), reducing endogenous cytokinin levels resulted in accelerated senescence (Masferrer et al., 2002). The components involved in cytokinin signalling is reported by Kieber and Skallar (2010) and Hwang et al. (2002). Among the genes characterised, only the receptor CKI1 and the response regulator ARR2 appear to be involved in regulating leaf senescence (Hwang et al., 2002).

Auxin, which regulates many aspects of plant growth and development, also influence leaf senescence, and is reported to delay leaf senescence (Misra and Biswal, 1980; Ellis et al., 2005).

The role of ethylene in senescence has been demonstrated by several studies. Both ethylene-insensitive mutants *etr1-1* and *ein2* show increased leaf longevities (Grbic and Bleecker, 1995; Johnson and Ecker, 1998) and antisense suppression of the tomato ACC oxidase resulted in delayed leaf senescence (John et al., 1995). Exogenously applied ethylene induces premature leaf senescence in *Arabidopsis* (Weaver et al., 1998). However, constitutive application of ethylene does not change the longevity of the leaves. Both *ctr1* mutants and *Arabidopsis* plants grown in the continuous presence of exogenous ethylene did not show premature senescence (Grbic and Bleecker, 1995). These studies suggest that ethylene does not directly regulate the onset of leaf senescence, but it acts to modulate the timing of leaf senescence (Grbic and Bleecker, 1995; Jing et al., 2002). The combined physiological and genetic studies by Buchanan-Wollaston et al. (2003) using *Arabidopsis* mutants exhibited a dual function of ethylene, both as an inducer and a repressor, in the induction of leaf senescence and that such a role of ethylene was differentially modulated by multiple genetic loci.

Salicylic acid (SA) is also involved in the regulation of this senescence. Its levels increase in senescing leaves, possibly accounting for the enhanced expression of *SAG* genes (Morris et al., 2000). Mutant analysis in *Arabidopsis* plants showed that the SA pathway has a very specific role in natural senescence, possibly in the final death phase or PCD (Morris et al., 2000; Buchanan-Wollaston et al., 2005).

Jasmonates (JAs) were proposed to play a regulatory role in leaf senescence. Early experiments, involving treating leaves or cell cultures with jasmonates, showed that a loss of chlorophyll was induced and the expression of photosynthetic-associated genes was suppressed (Creelman and Mullet, 1997). Jasmonates could rapidly induce the expression of chlorophyllase (Tsuchiya et al., 1999) and several *SAG*s (He et al., 2002). However, mutants that are impaired in JA biosynthesis and signalling (Berger, 2001), were not aberrant in phenotypic expression for leaf senescence, suggesting that jasmonates are not essential for leaf senescence. In addition, transgenic plants that either overexpress allene oxide synthase, jasmonic acid carboxyl methyltransferase, or underexpress lipoxygenase, did not show abnormal leaf senescence. Thus, molecular genetic analysis of jasmonate-related mutants did not generate any crucial link between jasmonate action and leaf senescence, and the role of jasmonates in leaf senescence is still a question of debate. It is possible that other factors might induce leaf senescence in the absence of JA.

Brassinosteroids could promote leaf senescence and mutants deficient in brassinosteroids showed altered leaf senescence indicating their involvement in leaf senescence (Clouse and Sasse, 1998; Yin et al., 2002). Nevertheless, a systematic study is needed to dissect the regulatory functions of these hormones in leaf senescence.

Abscisic acid (ABA) is a key hormone that mediates plant responses to environmental stresses. ABA levels rise in senescing leaves, and exogenously applied ABA induces the expression of several SAGs (Weaver et al., 1998), consistent with an effect on leaf senescence. Environmental stresses, including drought, high salinity, and low temperatures, have positive influences on leaf senescence and, under those conditions, leaf ABA contents rise. Concurrent with this increase (Gepstein and Thimann, 1980), genes encoding the key enzyme in ABA biosynthesis show greater expression (van der Graaff et al., 2006). Physiological analysis has shown that ABA (abscisic acid) could promote leaf senescence, but to date molecular genetic analysis has not generated a crucial link between ABA and leaf

senescence (Fedoroff, 2002). But there is an interaction and crosstalk between plant hormones, regulating the signaling network, which in turn regulates almost all processes in plants. This is likely the case in regulating leaf senescence by plant hormones.

4. Degradation of chloroplast protein during senescence

Protein synthesis and protein degradation are equally important for changes in the protein pattern and are of fundamental importance for the normal development, homeostasis and final death of a plant cell (Vierstra, 1996). Proteolysis in plants is a complex process involving many enzymes and multifarious proteolytic pathways in various cellular compartments (Sakamoto, 2006; Misra et al., 1991). ATP-independent and ATP-dependent proteolytic pathways are involved in plant proteolysis (Callis, 1995). It has been proposed that chloroplast proteins may be degraded by vacuolar proteases, via the ubiquitin pathway in the cytosol and also by the plastidial Clp system (Vierstra, 1996). The protein and RNA degradation parallels a loss in photosynthetic activity (Misra and Biswal, 1981a; 1982; Buchanan-Wollaston et al., 2003). The degradation products are transported out of the leaves to other parts of the plant, and so the senescing leaf continues to function as a source of nutrients to the whole plant. This nutrient salvage leads to the hydrolysis of macromolecules and subsequent remobilization requires a complex array of metabolic pathways. The initial stage of senescence symptoms is a breakdown in membrane structure within the chloroplasts, where more than 50% of the leaf protein and more than 70% of its lipids are present (Misra and Biswal, 1982; Misra and Misra, 1987; Hortensteiner and Feller, 2002). Chloroplasts are a major site of protein degradation during leaf senescence (Misra et al. 2002; Feller et al., 2008). Chloroplast degeneration is accompanied by chlorophyll degradation and a progressive loss of chloroplast proteins, e.g., ribulose bisphosphate carboxylase (Rubisco) and chlorophyll *a/b* binding protein (CAB) (Misra 1993a, b; Misra and Biswal, 1982b; Dilnawaz et al. 2001). The complete hydrolysis of proteins to free amino acids depends on the actions of several endo- and exopeptidases (Misra 1993a, b). Rubisco is the most abundant protein on earth and contributes a high percentage to the total leaf nitrogen remobilized to the developing parts of the plant by leaf senescence (Feller et al., 2008). Roberts et al. (2003) reported a serine protease activity in senescing wheat leaves for which Rubisco was a target protein. LSU (Large Subunit Rubisco) proteolysis is most likely catalyzed enzymatically by a metalloendopeptidase or a cysteine endopeptidase inside the chloroplasts (Thoenen et al., 2007) and/or non-enzymatically cleaved by reactive oxygen species (Dilnawaz et al., 2001; Nakano et al., 2006). Reactive oxygen species may directly cleave the plastid proteins or modify it in a manner making it more susceptible to proteolytic cleavage (Misra 1993a). Increased levels of reactive oxygen species during leaf senescence is reported (Tunc-Ozdemir et al., 2009; Misra et al., 2011a).

4.1 Regulation of chlorophyll degradation

Chlorophyll (chl), the most abundant pigment on earth, is a key component of photosynthesis required for the absorption of sunlight. A common and stepwise Chl degradation pathway in higher plants is elucidated by Hortensteiner (2006). Plant leaves generally change in color from green to yellow or red as a result of the breakdown of the green pigment chlorophyll (Chl) combined with carotenoid retention or anthocyanin accumulation, as a result of leaf senescence or accelerated cell death caused by various biotic or abiotic stresses (Matile et al.,

1999, Nooden et al., 1997). Chl catabolism is a multistep pathway. Chls in thylakoid membranes are degraded to nonfluorescent Chl catabolites and are accumulated in the vacuoles of senescing cells (Matile et al., 1988; Hortensteiner, 2006). There are three steps in Chl catabolism before the porphyrin ring is cleaved:

Step I - chlorophyllase converts Chl a into chlorophyllide a (Chlide a),

Step II - Mg-chelating substance converts Chlide a into pheophorbide a (Pheide a),

Step III - Pheide a oxygenase (PaO) converts Pheide a into red Chl catabolite.

Subcellular fractionation experiments show that chlorophyllase activity is present in the inner envelope membrane of chloroplasts (Matile et al., 1997). However, the substrate Chl, is tightly bound to the light-harvesting chlorophyll binding protein I (LHCPI) and II complexes in the thylakoid membranes. The spatial separation between Chlase and Chl has led to the hypothesis for a possible Chl carrier in the chloroplast stroma that shuttles between thylakoid and inner envelope membranes for Chl transport (Matile et al., 1997, 1999; Hortensteiner and Matile, 2004). Satoh et al. (1998) proposed the water-soluble chlorophyll protein (WSCP) as a feasible candidate for a Chl carrier. However, a recent report indicated that WSCP might act as a Chlide transporter during Chl synthesis in developing leaves rather than during Chl degradation in senescing leaves (Reinbothe et al., 2004). In higher plants, Chlorophyllase (CLH) genes encode soluble proteins that are predicted to localize in the cytoplasm, vacuole, or chloroplast stroma (Tsuchiya et al., 1999; Takamiya et al., 2000; Okazawa et al., 2006). In Arabidopsis thaliana, At CLH1 (At1g19670) encodes a putative cytosolic chlorophyllase and is upregulatedin response to stress and/or senescence-related hormones such as wounding, methyl jasmonate, and coronatine (Benedetti et al., 1998; Tsuchiya et al., 1999; Benedetti and Arruda, 2002). On the other hand, At CLH2 (At5g43860), which encodes a putative chloroplast chlorophyllase, is constitutively expressed at a low level throughout leaf development, and this expression is unaffected by either by stress or by senescence (Tsuchiya et al., 1999; Benedetti and Arruda, 2002). However, the gene(s) encoding the inner envelope membrane-bound chlorophyllase has not yet been identified, and it is still unknown which chlorophyllases are involved in the first step of Chl catabolism during leaf senescence. In this respect, the stay-green (also called nonyellowing) mutants isolated from several plants have been of great interest in elucidating the genetic and biochemical mechanisms of Chl breakdown during leaf senescence (Thomas and Smart, 1993; Thomas and Howarth, 2000).

The nonyellowing *sid* mutant of *F. pratensis* accumulates significant amounts of Chlide a and Pheide a in the senescing leaves and has no PaO activity (Roca et al., 2004), suggesting that Chl dephytylation by Chlases is suppressed by Pheide a accumulation in senescing leaves. In Arabidopsis, the PaO-impaired mutants, pao1 and AsACD1, which were induced by T-DNA insertion and antisense silencing of ACCELERATED CELL DEATH1 (ACD1) (Greenberg and Ausubel, 1993), respectively, maintained leaf greenness only during dark-induced senescence (Tanaka et al., 2003; Pruzinska et al., 2005). However, the decreased PaO activity in the mutants resulted in age- and light-dependent cell death in mature leaves, possibly due to the accumulation of the photodynamic Chl catabolite Pheide a (Pruzinska et al.,2005; Tanaka et al., 2003). Thus, high levels of Pheide a in the AsACD1 leaves did not exhibit persistent greenness during natural senescence (Tanaka et al., 2003). Another PaO-impaired mutant in maize (*Zea mays*), lethal leaf spot-1 (lls1), forms several necrotic spots that spread continuously until all of

the mature leaves are wilted and bleached (Gray et al., 2002). The red Chl catabolite reductase–impaired acd2 mutants in Arabidopsis also exhibit spontaneous cell death lesions in mature leaves (Mach et al., 2001). These defective phenotypes demonstrate that genetic lesions associated with the Chl catabolic pathway will ultimately result in cell death in green mature leaves, thus demonstrating that Chl catabolism is tightly regulated throughout plant development. Since the staygreen(Sgr) mutants do not show any age- and/or light-dependent cell death syndrome under natural growth conditions, it has been proposed that, during leaf senescence, the stay-green genes may encode the regulatory proteins triggering Chl catabolism rather than one of the Chl catabolic enzymes (Pruznska et al., 2005; Tanaka et al., 2003; Hortensteiner, 2006). It was recently reported that the stay-green y mutant in Festuca/Lolium forage plants also resulted from a frameshift mutation of a Sgr homolog (Armstead et al., 2006). Sgr is highly senescence-inducible and encodes a previously uncharacterized chloroplast protein whose amino acid sequence is extremely conserved in higher plants. The overexpression of Sgr in transgenic rice and the reduced expression of Sgr homologs in the Arabidopsis pao1 and acd1-20 mutants demonstrate that Chl degradation is regulated by Sgr at the transcriptional level and that Sgr transcription is repressed by either an increase of Pheide a or a lack of PaO activity in the senescing leaves.

5. Dark induced senescence in leaves

Leaf senescence is an active process regulated by exogenous and endogenous factors especially light quality and quantity (Nooden et al., 1996). Darkness is more commonly considered to be an inducer of senescence. Besides leaf age, darkness is more commonly considered to be an inducer of leaf senescence and induces loss of chlorophyll (Misra and Biswal, 1980, 1981), nucleic acid and protein (Misra and Biswal, 1982b), and alterations in the thylakoid membrane function such as photosystem I (PS I) and PS II activities (Biswal et al., 2001; Misra and Biswal, 1982a; Misra, 1993a). The expression of various photosynthesis associated genes was reduced (Misra, 1993b; Kleber-Janke and Krupinska, 1997; Weaver et al., 1998). The dark induced senescence is supposed to be be reversed by returning plants to the light, at least within a thresh hold dark treatment (Kleber-Janke and Krupinska, 1997). The *SAG* gene expression in response to both whole-plant darkness and natural senescence, showed varied responses, with some genes responding similarly to both treatments and others responding very differently (Kleber-Janke and Krupinska, 1997; Weaver et al., 1998). When whole plants were darkened, in contrast, by all criteria except loss of total protein and chlorophyll (which still occurred less quickly and less strongly than with individual leaf darkness), senescence was not induced (Weaver and Amasino,2001).

6. Regulation of senescence by cytokinins, sugers and light

During the process of leaf senescence, chlorophyll and photosynthetic proteins are degraded (Humbeck et al., 1996). There are several factors that can accelerate or delay this breakdown of the photosynthetic apparatus. Whereas the plant growth regulators ABA and ethylene accelerate the symptoms of senescence (Smart, 1994), exogenous application of cytokinins inhibits the degradation of chlorophyll and photosynthetic proteins (Richmond and Lang, 1957; Badenoch-Jones et al., 1996). Senescence is also delayed in transgenic plants producing cytokinin by expression of a bacterial gene encoding IPT, the enzyme catalyzing the first step of cytokinin synthesis (Smart et al., 1991; Gan and Amasino, 1995).

Sugar signaling has emerged as an important regulator of leaf senescence (Rolland et al., 2002). Several lines of evidence suggest that a high concentration of sugars lowers photosynthetic activity and induces leaf senescence (Quirino et al., 2000; Moore et al., 2003). Senescence is then triggered when those concentrations go above an acceptable level. Hexokinases are involved in sugar sensing in higher plants. Studies using their over expressors have demonstrated that increased hexokinase levels stimulate a rise in sugar content that is associated with reduced photosynthetic activity (Jang et al., 1997; Dai et al., 1999). One notable phenotype found in transgenic plants is accelerated leaf senescence, supporting the idea that lower photosynthetic activity may be related to premature leaf senescence via hexokinase. Moreover, a glucose-insensitive *Arabidopsis* mutant (gin2), with a lesion in one of the hexokinases shows delayed senescence (Moore et al., 2003). However, The *hysl (hypersenescencel)* mutant has increased sensitivity to exogenously applied sugars as well as an accelerated leaf senescence phenotype (Yoshida et al., 2002b). Therefore, one might suggest that an enhanced sugar signal in that mutant causes diminished photosynthesis and induces premature senescence, likely via hexokinase. Sugar-signaling pathways interact intimately with those signaling pathways regulated by hormones, e.g., auxin, cytokinin, or abscisic acid, during plant development. This is likely the case in the regulation of *Arabidopsis* leaf senescence. Such control through sugar signaling probably is also affected by other factors, such as nitrogen status and developmental stage. Integration of these factors into a senescence program might be important to the proper regulation of timing for its onset and progression.

The photoautotrophic nature of plants makes them fundamentally different from animals. Their energy input depends on the available photosynthetic activity, light and CO_2 and altering the available sources could substantially change the process of leaf senescence. Miller et al. (1997) found that elevated CO_2 could accelerate the shift of leaf development from the photosynthetic activity increase phase to the decrease phase. Ludewig and Sonnewald (2000) subsequently showed that this was caused by the earlier onset of leaf senescence. Leaf senescence was also examined in plants with reduced available sources. In *Rubisco* antisense tobacco plants, less dry weight and chlorophyll content was achieved than the wild type at maturity, while the leaf ontogeny was not altered (Miller et al., 2000). The most striking feature of the *Rubisco* antisense plants is that the senescence was markedly prolonged resulting in extended longevity. This pattern is similar to one of the stay-green mutants described in pea (Thomas and Howarth, 2002). More recently, the *Arabidopsis* delayed leaf senescence mutant *ore4-1*, was shown to contain a T-DNA insertion in the plastid ribosomal small subunit protein 17 (*PRPS17)* gene (Woo et al., 2002). The *ore4-1* mutants achieved less dry weight and contained less chlorophyll contents as in the *Rubisco* antisense plants, and more importantly, the photosynthetic system I activity of the *ore4-1* mutants was impaired. These results suggest that disruption of *PRPS17* resulted in reduced chloroplast function and energy input, perhaps mimicking the effect of calorie restriction in animals. Thus, increased energy input (mimicking over feeding in animals?) could accelerate leaf senescence, whereas reduced energy input had an opposite effect. It has been proposed that leaf senescence is initiated when photosynthetic activity drops below a certain threshold level (Hensel et al., 1993). This threshold could be related to leaf sugar levels. Indeed, leaf soluble sugar content increases with leaf age, and growth on media supplemented with sugars could repress photosynthesis associated gene (*PAG)* transcription and translation (Dijkwel et al., 1997; Jang et al., 1997; Wingler et al., 1998).

Sugars could specifically inhibit the expression of several *SAG*s associated with dark induction (Fujiki et al., 2001). However, in *SAG12-ipt* (isopentenyl transferase) transgenic tobacco the sugar levels were not different from *SAG12-GUS* plants, although senescence in the former one was substantially delayed (Ludewig and Sonnewald, 2000). In the senescent leaves the soluble sugars showed higher levels than in the non-senescent leaves presumably due to the breakdown of chloroplast and cell wall compounds (Quirino et al., 2000). This suggests that increased sugar levels are a consequence rather than a signal to initiate senescence. Exogenous sugars also had different effects on the expression profile of *SAG*s. While enhancing the expression of *SAG21* and *SAG13*, sugars inhibited the expression of *SAG12* (Noh and Amasino, 1999; Xiao et al., 2000). Taken together, the absolute level of sugars appears not to be directly involved in the regulation of leaf senescence. On the other hand, compelling evidence shows that sugar sensing and signalling can influence senescence. In *Arabidopsis* plants overexpressing sense and antisense hexokinase genes (*AtHXK1* and *AtHXK2*), the greening process and the expression profile of *PAG*s and *SAG21* were directly correlated with *AtHXK* expression levels (Jang et al., 1997; Xiao et al., 2000). Similar results were observed in transgenic tomato plants overexpressing *Arabidopsis AtHXK1* (Dai et al., 1999). The *gin2* mutant that has a lesion in the *AtHXK1* gene shows delayed leaf senescence as well as reduced glucose sensitivity (Quirino et al., 2000; Rolland et al., 2002). The *cpr5* mutant that was originally isolated based on the altered pathogen resistance was shown to have sugar hypersensitivity and early leaf senescence (Bowling et al., 1997; Yoshida et al., 2002a). Thus, altered energy intake or sensing can substantially influence senescence. However, more studies are needed to elucidate the precise molecular mechanisms. It is known that sugars can interact with several distinct signalling pathways such as ABA, ethylene, light and cytokinins, all of which are implicated in regulation of leaf senescence (Smeekens, 2000; Rolland et al., 2002). The effect of sugars on leaf senescence may depend on these interactions.

Leaf senescence is influenced by environmental conditions, including light, temperature, ion, salts, nutrient or water stress, or oxidative stresses induced by ozone or UV-B and biotic stresses like pathogen infection. Low light intensities or darkness results in the reduced expression of light-dependent genes and the disappearance of photosynthetic proteins and chlorophyll (Thomas, 1978). Exposure to extremely high or low temperatures, pathogen attack, or water/nutrient deficiency can also trigger leaf yellowing. Thus, parts of the signaling pathways that are associated with environmental stresses would be predicted to regulate leaf senescence. Expression profiles of 402 potential stress-related genes that encode known or putative transcription factors from *Arabidopsis* have been monitored in various organs, at different developmental stages, and under several biotic and abiotic stresses (Chen et al., 2002). Among the 43 transcription factor genes that are reportedly induced during senescence, 28 are also induced by stress treatment, suggesting extensive overlapping responses. Downstream genes for senescence-enhanced transcription factors might play a role either in executing leaf senescence or in protecting the cellular functions required for proper progression or completion of that senescence.

Light, perceived by a variety of photoreceptors, affects developmental processes over the entire life span, and may also play a role in leaf senescence (Cherry et al., 1991; Thiele et al., 1999). For example, transgenic plants over expressing phytochrome A *(PhyA)* or phytochrome B *(PhyB)* exhibit greater longevity. Although a mechanism for delayed

senescence has not yet been proposed, one cause might be a slower chlorophyll degradation and leaf yellowing in these mutants. Since phytochrome acts as the light receptor for the expression of many photosynthetic genes, a lower red/far-red ratio reaching the lower leaves of a plant can also accelerate the senescence of these leaves (Rousscaux et al., 1996). In nonsenescent leaves sugar accumulation can lead to a decline in chlorophyll and photosynthetic proteins (Krapp and Stitt, 1994). Glc and Suc repress the transcription of photosynthetic genes, probably acting via hexokinase as a sugar sensor (Jang et al., 1997). The involvement of sugar-mediated repression of genes in the regulation of natural senescence is less clear (Feller and Fischer, 1994). The concentration of leaf sugars can increase during leaf senescence and accumulation of sugars, induced by removal of sinks or phloem interruption, can both accelerate and delay senescence (Frohlich and Feller, 1991). The response of leaves to the accumulation of sugars must therefore also depend on other factors, such as the C:N status of the leaf (Paul and Driscoll, 1997), light (Dijkwel et al., 1997) and plant growth regulators (Koch, 1996). For example, it has been suggested that cytokinin, in addition to delaying senescence, could block some of the responses to sugars (Jang et al., 1997). The interactions of cytokinins, light, and sugars during senescence in transgenic tobacco (*Nicotiana tabacum* L.) plants with autoregulated synthesis of cytokinin (Gan and Amasino, 1995). The transgenic tobacco plants express the gene for IPT under control of the senescence-specific SAG 12 promoter (Lohman et al., 1994). This promoter is activated at the onset of senescence, leading to the synthesis of cytokinin. Because of the inhibition of senescence by cytokinin, the promoter is actively attenuated. This results in an autoregulatory loop, preventing the overproduction of cytokinin and confining expression solely to those tissues that have initiated senescence. Apart from a delay in senescence, these plants therefore develop normally (Gan and Amasino, 1995). In cotyledons of curcurbits the synthesis of HPR is induced by cytokinin (Chen and Leisner, 1985; Andersen et al., 1996) and light (Bertoni and Becker, 1993), and the activity of HPR decreases during senescence (De Bellis and Nishimura, 1991).

It was shown that leaves of soybean (Guiamet et al., 1989) and sunflower (Rousseaux et al., 1996) senesced more quickly when the red:far-red ratio of the light they received was decreased, and that far-red light induces chlorophyll loss in tobacco leaves (Rousseaux et al., 1997). It also has been shown that tobacco and overexpressing oat phytochrome A display both delayed leaf senescence (Cherry et al., 1991) and an inhibited response to the senescence promoting effects of far-red light (Rousseaux et al., 1997). Weaver and Amasino (2001) examined a *hy2/hy3* phytochrome double mutant line, in which there is no phytochrome B and low levels of all other phytochromes (Parks and Quail, 1991; Somers et al., 1991), and observed it to behave much like wild-type controls, after both whole-plant and individual leaf dark treatments. One should note, however, that if phytochrome is involved in inhibiting senescence it might not be surprising that covered leaf senescence would continue to occur in phytochrome mutants, and in some instances might occur more strongly.

7. Senescence-associated PCD

Senescence is a complex, highly ordered process, during which plant organs undergo a series of biochemical and physiological changes ultimately resulting in the death of the organ (Smart, 1994; Buchanan-Wollaston, 1997). However, until late in senescence the process requires cell viability and is often reversible (Thomas *et al*., 2003). There has been

some debate about the degree of overlap of senescence and PCD (Thomas *et al*., 2003; van Doorn & Woltering, 2004). van Doorn & Woltering (2004) identified various types of overlap in senescence and PCD.

i. Total overlap: Senescence and PCD overlap is complete and are synchronous (Noodén, 2004; van Doorn & Woltering (2004, 2008).
ii. No overlap: PCD either operated as the final instalment of senescence, or equally plausible, operated following senescence (Delorme *et al*., 2000; Thomas *et al*., 2003). van Doorn & Woltering (2008) defined PCD as 'the process that leads to the moment of death and the degradation that goes on after this moment'. Senescent cells are being actively recycled, which means that the cells are subjected to nucleases, proteases and photosynthetic breakdown along with various other senescent-related stresses. Normally one might expect that a cell which is subjected to this type of stress would activate PCD. However, in order to complete senescent recycling, PCD may have to be actively suppressed during the senescence process and only activated when recycling has been completed. For example, Bax inhibitor-1 (BI-1) has been shown to suppress PCD in both plant and animal cells (Watanabe & Lam, 2006). Bax inhibitor-1 is upregulated during harvest-induced senescence in broccoli (Coupe *et al*., 2004). Another PCD suppressor, defender against apoptotic death (DAD1), is upregulated during leaf senescence (Dong *et al*.,1998), whereas it is down regulated before the onset of apoptotic like cell death in shorter-lived petals of pea (Orzaez & Granell,1997), *Alstroemeria* (Wagstaff *et al*., 2003) and gladiolus(Yamada *et al*., 2004). It is hypothysed that senescence may indeed be a rich source of PCD genes, but genes involved in suppression, rather than activation, of the process, and indeed activation of PCD, may be a result of senescence having terminated and consequently a cessation of transcription of PCD-suppressing genes and gene products.

8. Altered membrane lipase expression delays leaf senescence

Membrane deterioration leading to leakiness and loss of selective permeability is an early and ubiquitous feature of senescence (Thompson et al, 1998). It affects both the plasma membrane and intracellular membranes, and results in loss of ionic and metabolite gradients that are essential for normal cell function (Paliyath and Thompson, 1990) initiating programmed cell death. As the study of membrane lipids in green leaves is relatively cumbersome, the informations regarding senescence induced changes in membrane lipids are deried from floral senescence and PCD. Lipases are involved in leakiness of cellular membranes in senescing plant tissues (Borochov et al, 1982), resulting in an increase in the nonesterified/esterified fatty acid ratio (Thompson et al,1998). Moreover, the onset of membrane leakiness appears to be attributable, at least in part, to lateral phase separations of non-esterified fatty acids within the plane of the membrane bilayer. The resulting mixture of lipid phases causes the membranes to become leaky because of packing imperfections at the phase boundaries (Thompson et al, 1998). In addition, de-esterified polyunsaturated fatty acids in senescing membranes serve as substrates for lipoxygenase and ensuing lipid peroxidation, and the formation of peroxidized lipids in membranes also contributes to the onset of leakiness (Thompson et al, 1998). The abundance of the lipase mRNA increases just as carnation flowers begin to senesce, and expression of the gene is also induced by treatment with ethylene (Hong, 2000).

During leaf senescence, reactive oxygen species (ROS) and oxidative damage increases, whereas the levels of antioxidant enzymes such as SOD, catalase, and ascorbate peroxidase decreases (Orendi et al.,, 2001; Munne-Bosch and Alegre, 2002). Leaf senescence and the expression of various *SAG*s were promoted in old leaves upon exposure to UV-B or ozone, which are known oxidative damage inducing treatments (Miller et al., 1999; John et al., 2001). Mutant analysis and studies on transgenic plants provided a more straightforward support for the role of ROS in senescence (Orvar and Ellis, 1997; Willekens et al., 1997). Thus the molecular analysis substantiates the direct involvement of ROS in leaf senescence. ROS have a tight relationship with membrane and lipid dynamics since the membrane associated NAD(P)H oxidases can sense both endogenous and exogenous stresses and are one of the major generators of ROS (Mittler, 2002). The involvement of lipid metabolism in leaf senescence was demonstrated (He and Gan, 2002). Lipids are produced by fatty acid biosynthesis pathways, hence mutations in this pathway were also shown to change leaf senescence (Mou et al., 2000; Wellesen et al., 2001). Thus, ROS-induced membrane shuffling and lipid metabolism is not a passive wear and tear process but actively involved in leaf senescence. There is an intrinsic link between oxidative damage and leaf senescence, and the free radical theory of ageing seems to apply to plant senescence. Senescent tissues are stressed and subjected to gradually increasing oxidative damage. But, leaf cells continue to functionally operate transcriptional and translational activities along the progression of leaf senescence. So, plants have inherent mechanisms to safe guard the genome stability until the last stage of leaf senescence i.e. through PCD. (Liu et al. 2007).

9. Senescence in plants: Conserved strategies and novel pathways

Senescence is a universal phenomenon in living organisms and in higher plants it is manifested by the senescence of leaves. Constituting the last part of leaf development, leaf senescence has evolved as an indispensable process to maximise the reutilization of nutrients accumulated in the senescing leaves (Leopold, 1961; Bleecker, 1998). Molecular dissection of senescence process will provide information about the regulation of developmental cell death in plants or leaves, which can be utilized in crops for agricultural productivity. The expression of *SAG*s are up-regulated during senescence. To date over 100 *SAG*s have been identified in diverse plant species, and the list of *SAG*s is still increasing. Their expression profiles have been examined during development and under various induction conditions (Smart, 1994; Buchannan-Wollaston, 1997; Gan and Amasino, 1997; Nam, 1997). Many clones of *SAG*s showing up-regulation of their expression during senescence are also been reported to be overexpressed with abitic and biotic stress. Plant leaf senescence is modulated at a large array of genetic loci (Bleecker and Patterson, 1997; Buchanan-Wollaston, 1997; Quirino et al., 2000). However, a single gene mutation can substantially alter the lifespan in yeast, worms, fruit flies and some mammals (Kirkwood and Austad, 2000). Emerging evidences allowed the analysis of pathways involved and to compare the molecular strategies and the conserved nature of the senescence processes in plants and animals.

10. Nitric oxide evolution during leaf senescence

More recently, NO, a mediator of various plant developmental and (patho) physiological processes (Neill et al.2003; Crawford and Guo 2005; Mur et al. 2006), has been implicated in plant senescence and maturation. For instance, the temporal progress of fruit maturation

and floral senescence is associated with a significant decrease in NO emission, and application of NO donating compounds retards flower senescence and extends the post-harvest life of fruits and vegetables (Leshem et al. 1998). Similarly, NO emission from *Arabidopsis* plants decreases significantly when plants mature and leaves start to senesce (Magalhaes et al. 2000). In addition, exogenous NO counteracts the promotion of leaf senescence caused by ABA and methyl jasmonate in rice (Hung and Kao, 2004).

To date, two major biochemical means of NO production have been identified in plants. On one hand, NO can be produced through enzymatic or non-enzymatic reduction from nitrite (Stöhr *et al*. 2001; Rockel *et al*. 2002; Bethke *et al*., 2004). In fact,a major source of NO in plants originates from nitrite mediated by the action of nitrate reductase (Yamasaki and Sakihama 2000; Kaiser *et al*. 2002).

Interestingly, several factors, among them cytokinin, light and nitrate treatment, do simultaneously stimulate the expression or activity of NR (Crawford 1995; Yu, Sukumaran and Márton 1998), enhance the *in planta* production of NO (Magalhaes *et al*. 2000; Tun et al. 2001; Planchet *et al*. 2006) and retard the progress of plant senescence (Smart 1994). On the other hand, NO can be generated by NOS from L-arginine, and a corresponding plant NOS gene (*AtNOS1*) has been cloned and characterized in *Arabidopsis* (Guo *et al*., 2003).The AtNOS1 protein is localized to mitochondria, and is involved in ABA-induced stomatal closure, the control of flowering and defense responses towards bacterial lipopolysaccharide elicitors (Guo *et al*. 2003; He *et al*. 2004; Zeidler *et al*. 2004; Guo and Crawford 2005). Moreover, NOS activity appears to represent one enzymatic means to influence plant senescence, as dark-induced leaf senescence occurs more rapidly in *Atnos1* knockout mutants compared with WT plants (Guo and Crawford 2005). In addition, a NOS-like activity of pea peroxisomes is down-regulated during the senescence process in pea leaves (Corpas *et al*. 2004).

In an attempt to study the (patho)physiological effects of reduced endogenous NO levels in plants, Mishina et al.,(2007) expressed a bacterial NOD under the control of an inducible promoter in *Arabidopsis* and observed that the resulting NO-deficient plants undergo a senescence-like process several days after activation of the NOD. They showed that this NOD-induced senescence process shares many similarities with natural senescence at the molecular level, and report effects of exogenous NO treatment on the progression of NOD- and dark-induced senescences andsupport the hypothesis that NO acts as a negative regulator of leaf senescence.

Arabidopsis Col-0 plants expressing the bacterial flavohemoglobin Hmp (Zeier *et al*. 2004) that functions as a NOD in *Escherichia coli*, converting NO to nitrate by using NAD(P)H and O2 (Poole and Hughes 2000). Leaf yellowing as a consequence of chlorophyll degradation represents the first visible symptom of senescence (Quirino *et al*. 2000). During natural senescence and senescence induced by artificial treatments, like shading of detached leaves, many photosynthetic genes are actively down-regulated (Lohman *et al*. 1994). When following the expression patterns of the typical photosynthesis-related genes *CAB* and *RBCS* [small subunit of ribulose 1 ·5-bisphosphate carboxylase/ oxygenase (Rubisco)] were down-regulated after NOD induction and this decline in expression of photosynthetic genes just occurred before visible signs of leaf yellowing (Mishina et al., 2007).

In addition to the down-regulation of photosynthesis, leaf senescence is characterized by an increase in expression of a multitude of genes that are often referred to as ***SAGs*** (Buchanan-

Wollaston 1997; Gepstein *et al*. 2003). Recently, Vladkova et al. (2011) reported that NO directly affects photosynthesis by enhancing it at low concentrations and is inhibitory at higher concentrations. This concentration dependent regulation of photosynthesis could inturn regulate leaf senescence (Misra et al. 2010a, b, 2011b)

Visible leaf yellowing in response to NOD expression occurred faster and was more pronounced in old than in young Hmp leaves. The findings that the expression of a NO degrading enzyme in *Arabidopsis* and the concomitant decrease in plant NO levels (Zeier *et al*. 2004) lead to a yellowing phenotype and changes in gene expression similar to senescence. These findings suggest that the senescence-like effect in Hmp plants was a result of NO deficiency that could be compensated by external NO. Induced expression of the *E. coli* flavohemoglobin Hmp in *Arabidopsis*, an enzyme functioning as a NOD (Poole and Hughes 2000), reduced both the *in planta* detection of NO as well as the emission of NO from plants, and furthermore increased the NO degrading capacity of leaf extracts (Zeier *et al*. 2004). The cysteine protease gene *SAG12* is known to be expressed exclusively during senescence, and its expression is therefore used as a senescence-specific marker (Zimmermann et al., 2006). The up-regulation of *SAG12* during the NOD-induced senescence phenotype indicates a high similarity of the process to natural senescence.

Reduction of nitrite by NR represents a major enzymatic plant NO source (Yamasaki and Sakihama 2000; Kaiser *et al*. 2002). Consequently, factors like nitrate feeding, high light treatment or cytokinin application, all of which stimulate NR activity (Crawford 1995; Yu *et al*. 1998), were able to enhance NO formation in plants (Magalhaes *et al*. 2000; Tun *et al*. 2001; Planchet *et al*. 2006). The nitrate remobilization out of the senescing leaves might cause a perturbation in the NO synthesis and regulate the process of senescence and associated PCD.

11. Regulation of leaf senescence by nitric oxide

A negative regulatory role of NO in plant senescence and maturation is studied extensively (Leshem *et al*. 1998; Magalhaes *et al*. 2000; Hung and Kao 2004; Guo and Crawford 2005). The findings that leaf yellowing, the down-regulation of *CAB* and SAG expression during NOD-induced senescence are attenuated by exogenous application of NO. During natural ageing of *Arabidopsis*, plant NO emission decreases continuously and reaches a minimum value during senescence (Magalhaes *et al*. 2000). Considering the processes observed in NO-deficient Hmp and *Atnos1* plants, we speculate that during leaf development, NO levels might fall below a certain threshold that in turn contributes to the induction of natural senescence.

Thus, application of NO releasing chemicals or NO gas extended the post-harvest life of fruits and vegetables and retarded the senescence of flowers (Leshem *et al*. 1998). Further, NO donors counteracted methyl jasmonate and ABA promoted senescence of rice leaves (Hung and Kao 2003, 2004), and attenuated dark-induced leaf senescence in *Arabidopsis Atnos1* mutants (Guo and Crawford 2005). The dose-dependent action of NO as a double-edged sword (Colasanti and Suzuki 2000), providing anti-senescent properties at lower and damaging effects at higher concentrations.

The integration and balance of internal and external factors is thought to be important in controlling the induction of leaf senescence (Yoshida 2003). Leaf age represents a significant internal variable influencing senescence. For instance, Weaver and Amasino (2001) have shown that dark induced senescence in individual leaves occurs more rapidly and strongly

in older leaves than in younger ones. The older plants exhibit lower NO emission than young ones (Magalhaes *et al*. 2000).

NO emission is generally much more pronounced in light-situated plants as compared with darkened plants, and higher light intensities give rise to stronger NO emission signals than lower light levels (Magalhaes *et al*. 2000; Planchet *et al*. 2006). Similarly, nitrate-grown plants emit considerably higher amounts of NO than plant grown on ammonium (Planchet *et al*. 2006), and cytokinins give rise to increased NO releases in plant cell cultures (Tun *et al*. 2001). Light, nitrate and cytokinin are thus capable to stimulate endogenous NO production, and an internal NO generation caused by the previous treatments may have consequently counter balanced the NO diminishing action of NOD in Hmp plants. Considering the postulated antisenescent properties of NO, this in turn would explain the observed attenuation of NOD-induced senescence. NR may play a key role in mediating these effects, as NR expression and activity are known to be positively regulated by the three treatments applied (Crawford 1995; Yu *et al*. 1998), and at the same time, NR represents a major NO source in plants (Yamasaki and Sakihama 2000; Kaiser *et al*. 2002).A similar scenario would be feasible in plant development, during which light conditions, nitrogen nutrition and other environmental factors could be integrated through cytokinin action, NR activity and NO levels to influence the regulation of natural senescence.

NO has been recognized to possess both pro- and antioxidant effects in plants (Misra et al. 2010a, b, 2011), and this antagonism may be based on the relative ratios of ROS and NO levels in different physiological situations. By inhibition of antioxidant enzymes like catalase and ascorbate peroxidase, NO may contribute to elevated ROS levels and oxidative stress under certain circumstances (Clark *et al*. 2000). During the oxidative burst, NO ensures prolonged H2O2 levels at the site of pathogen challenge. Contrastingly, NO has been shown to act as an antioxidant in other situations. NO donors protect from oxidative damage caused by methylviologen herbicides, and counteract ROS-mediated programmed cell death in barley aleurone layers (Beligni and Lamattina 1999; Beligni *et al*. 2002).

During ABA- and jasmonate-induced senescence in rice, NO-releasing substances prevent an increase in H2O2 levels and lipid peroxidation (Hung and Kao, 2004). Accelerated dark-induced senescence in *Atnos1* mutants is accompanied with increased ROS levels and protein oxidation (Guo and Crawford 2005). As a free radical, NO reacts with superoxides in a diffusion limited reaction to form peroxynitrite (Huie and Padmaja 1993), and a subsequent fast isomerization of this toxic compound to a harmless end product like nitrate represents a possible mechanism to reduce ROS levels and cell damage through oxidative stress.

Although oxidative stress and ozone application induce various SAGs, these treatments do not initiate expression of the specific senescence marker *SAG12* (Miller *et al*. 1999; Navabpour *et al*. 2003).Thus, ROS elevation is not sufficient to trigger a full and coordinated execution of the natural senescence programme, and attenuation of *SAG12* expression by NO must therefore occur by ROS independent mechanisms. The plant hormone ethylene is capable of promoting senescence in plants (Smart 1994; Grbic and Bleecker 1995), and ethylene levels rise when plants start to senesce (Aharoni *et al*. 1979; Magalhaes *et al*. 2000). Considering the negative correlation of ethylene and NO emission during plant ageing (Magalhaes *et al*. 2000) and the up-regulation of ACC synthase during NOD induced senescence, it is plaussible that falling NO levels also contribute to senescence regulation by initiating ethylene biosynthesis.

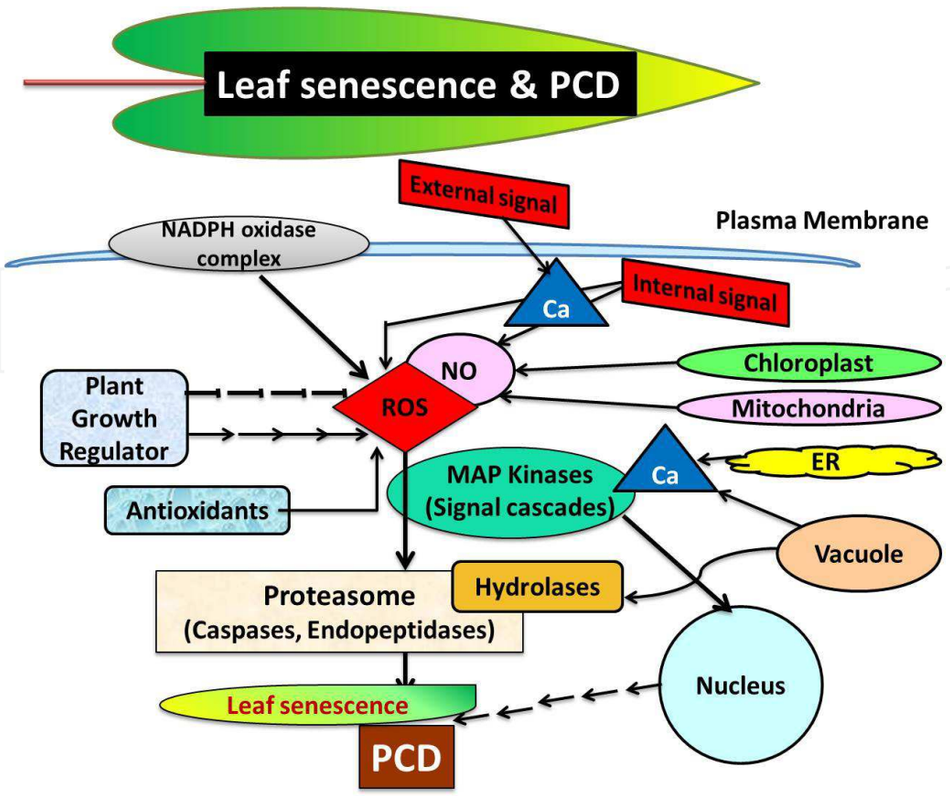

Fig. 1. A schematic diagram of the process and control of leaf senescence and the associated programmed cell death (PCD). The external and internal signals regulating leaf senescence and PCD is shown. The compartmentation of and the origin of different signals or signaling molecules are shown in the right panel. The central panel indicates the regulatory pathway. The leaft panel shows the physiological and biochemical regulatory mechanism for the control of various signaling and oxidative network pathways during leaf senescence or PCD in plants.

On one hand, NO can be produced through enzymatic or non-enzymatic reduction from nitrite (Misra et al. 2010a, b, 2011b and references there in). In fact, a major source of NO in plants originates from nitrite mediated by the action of nitrate reductase. Interestingly, several factors, among them cytokinin, light and nitrate treatment, do simultaneously stimulate the expression or activity of NR (Misra et al. 2010a, b, 2011b), enhance the *in planta* production of NO and retard the progress of plant senescence. On the other hand, NO can be generated by NOS from L-arginine, and a corresponding plant NOS gene (*AtNOS1*) has been cloned and characterized in *Arabidopsis* (Guo et al., 2003). The AtNOS1 protein is localized to mitochondria, and is involved in ABA-induced stomatal closure, the control of flowering and defense responses towards bacterial lipopolysaccharide elicitors (Zeidler *et al*. 2004; Guo and Crawford 2005). Moreover, NOS activity appears to represent one enzymatic means to influence plant senescence, as dark-induced leaf senescence occurs more rapidly in *Atnos1*knockout mutants compared with WT plants (Guo and Crawford 2005). In addition, a NOS-like activity of pea peroxisomes is down-regulated during the senescence process in pea leaves (Corpas *et al*. 2004).

12. Conclusion and future perspectives

This review focuses on the physiological, biochemical and molecular aspects of leaf senescence. Studies on these aspects are extensive and there is an wealth of knowledge on the senescence processes in plants. Although senescence and programmed cell death in animal tissue is well established, however the temporal and spatial correlation between these two events in plants and leaves are not well defined. This review tried to make a synthesis of the upto date information regarding these two aspects and bring about a common mechanism for both processes being regulated through a network of inter-related regulatory network such as reactive oxygen species and kinase caskades (Fig. 1). Also, the possible pathways of synthesis, cellular compartmentation and regulation of NO during leaf senescence and/or PCD is illucidated. However, this is the beginning of a new era of NO signaling in leaf senescence and PCD. The journey starts now for the elucidate of the mechanism of NO signaling in leaf senescence and PCD in plants.

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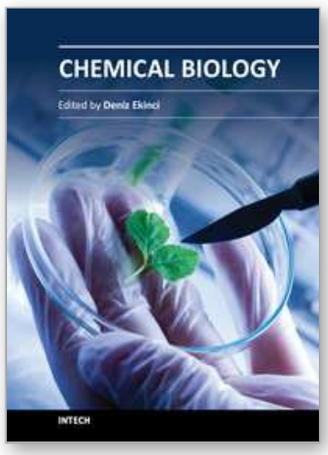

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Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

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