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The Role of Oxidative Metabolism in the Regulation of Leaf Senescence by the Light Environment

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ABSTRACT

Senescence is a genetically controlled process whose regulation depends on many factors. Leaf senescence begins with the catabolism of chlorophyll and macromolecules such as proteins and membrane lipids, a process that is accompanied by the alteration of organelles and, ultimately, the breakdown of nuclear components. In annual plants, reproduction is the main event that triggers leaf senescence. However, several environmental factors may initiate and/or modulate it both in the vegetative as well as the reproductive stages. An important exogenous factor is light, and both its senescence-inhibiting or -promoting qualities are described. Both the darkening of individual leaves as well as exposure to high light intensities can induce senescence, and it has been proposed that changes in the redox status of cells associated with the overproduction of reactive oxygen species (ROS) might be the basis of the senescence symptoms. In dense stands, basal leaves frequently senesce before anthesis, and there is evidence that this phenomenon is regulated by changes in light quantity and quality. While models about putative signalling pathways leading to either acclimation or to cell death involving the accumulation of ROS in response to excess light have been recently developed, less information is available with regards to the biochemistry of leaf senescence induced by changes in light spectral quality. In the present review we summarize a series of studies that have contributed to the view that changes in either light quantity or quality may modulate leaf senescence through signals derived from oxidative metabolism.

Keywords: leaf senescence, light intensity, light spectral quality, oxidative stress, programmed cell death, reactive oxygen species
Abbreviations: CAT, catalase; **EEE**, excess excitation energy; **FR**, far red; **MAPK**, mitogen activated protein kinase; **PCD**, programmed cell death; **R**, red; **ROS**, reactive oxygen species

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INTRODUCTION

Senescence is a genetically controlled process whose regulation depends on many factors. Leaf senescence begins with the catabolism of chlorophyll and macromolecules such as proteins and membrane lipids, a process that is accompanied by the alteration of organelles and, ultimately, the breakdown of nuclear components. In annual plants, reproduction is the main event that triggers leaf senescence. However, several environmental factors may initiate and/or modulate it both in the vegetative as well as the reproductive stages. An important exogenous factor is light, and both its senescence-inhibiting or -promoting qualities are described. Both the darkening of individual leaves as well as exposure to high light intensities can induce senescence, and it has been proposed that changes in the redox status of cells associated with the overproduction of reactive oxygen species (ROS) might be the basis of the senescence symptoms. In dense stands, basal leaves frequently senesce before anthesis, and there is evidence that this phenomenon is regulated by changes in light quantity and quality. While models about putative signalling pathways leading to either acclimation or to cell death involving the accumulation of ROS in response to excess light have been recently developed, less information is available with regards to the biochemistry of leaf senescence induced by changes in light spectral quality. In the present review we summarize a series of studies that have contributed to the view that changes in either light quantity or quality may modulate leaf senescence through signals derived from oxidative metabolism.

LEAF SENESCENCE AND PROGRAMMED CELL DEATH

Leaf senescence is an integral part of plant development. During this process nitrogen and other key nutrients are recycled to other plant parts and used to support new vegetative growth or the formation of reproductive structures and storage organs. Autumnal senescence of deciduous trees is perhaps the best known example of leaf senescence. In most studied species reduction in the photoperiod is the main environmental signal triggering this process (e.g. Lee *et al*.

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2003; Kukavica and Jovanovic 2004; Keskitalo *et al*. 2005). Tissue yellowing and protein degradation due to chlorophyll and chloroplast breakdown are among the most prominent features of senescing leaves. During the senescence process, chloroplasts differentiate into gerontoplasts that lack stacked thylakoid membranes but accumulate electron-dense lipid bodies (plastoglobuli) containing high levels of carotenoids and carotenoid degradation products. Depending on the species and environmental conditions (particularly excess light), anthocyanins also accumulate giving rise to dark-reddish colours (Lee *et al*. 2003; Keskitalo *et al*. 2005).

Chlorophyll breakdown is a multi-step process whose most remarkable feature is the oxygenolytic opening of the porphyrin macrocycle, catalized by the joint action of two enzymes: phaeophorbide *a* oxygenase (PaO) and red chlorophyll catabolite reductase (RCCR) (Hörtensteiner 2004). Even though both PaO mRNA and protein may be present in green tissues, the enzyme activity is mostly restricted to the senescence phase. On the contrary, the activity of other enzymes of the chlorophyll degradation pathway like chlorophyllase (catalizing the removal of the phytol group), Mg dechelatase or RCCR are constitutive (Langmeier *et al*. 1993; Hörtensteiner 2004). Mutants defective in the activity of either PaO (e.g. Gray *et al*. 1997) or RCCR (Greenberg *et al*. 1994; Mach *et al*. 2001) develop cell death lesions in their leaves in an age-dependent manner. Both types of mutants accumulate photoreactive chlorophyll catabolites upon dark induction of leaf senescence, and this phenomenon positively correlates with cell death progression of the respective mutants (Pružinská *et al*. 2003). The phenotype of these mutants show lesions similar to those induced in defense reactions to pathogens. Moreover, in maize PaO defective mutants lesion formation was shown to be light dependent (Gray *et al*. 2002). *Festuca* and *Lolium* plants homozygous for a stay-green mutation, also accumulate dephytylated, more polar derivatives, largely chlorophyllide and phaeophorbide, during senescence (see Thomas *et al*. 2002 and references therein). The phenotype of these mutants is also consistent with a PaO knockout. While chlorophyll *a* and total carotenoid levels as well as specific membrane (largely light-harvesting and reaction centre) proteins are much more stable in these and other (e.g. Bachmann *et al*. 1994) stay-green mutants, degradation of Rubisco and other proteins is close to normal. Hence, even though chlorophyll degradation is not very important for N recycling, is important in the prevention of the accumulation of toxic photodynamic intermediates during senescence, and is the most frequently used indicator of this process.

Senescence is not simply death or necrosis, neither merely a passive aging process because it requires new gene transcription and is subject to post-transcriptional and post-translational regulation (e.g. Gan and Amasino 1997; Noodén *et al*. 1997; Thomas *et al*. 2003). The function of many genes that are typically enhanced during senescence, referred as SAGs (senescence-associated genes) (Buchanan-Wollaston 1997; Quirino *et al*. 2000; Lim *et al*. 2003), is not always easy to establish. When the mRNA expression profiles of 402 potential transcription factors were monitored in *Arabidopsis* plants at different developmental stages and under various biotic and abiotic stresses, among the 43 transcription factor genes that were induced during senescence, 28 were also induced by stress treatments, suggesting that there is extensive overlap in the responses to leaf senescence and stress (Chen *et al*. 2002). The genes associated to senescence in this species have been grouped in six categories (Lim *et al*. 2003): Class 1; genes that control the developmental aging process. Class 2; genes that control other endogenous biological processes in plants in addition to leaf senescence. Class 3; genes that affect senescence in response to environmental factors. Class 4; regulatory genes that upregulate senescence-associated activities or downregulate cellular-maintenance activities. Class 5; genes that are suggested to be involved in the degradation process of senescence regulatory factors. Class 6; downstream genes that are involved in executing the senescence process, such as genes for cellular disintegration and nutrient recovery. Classes 1 to 5 are primarily involved in the initiation and/ or propagation of senescence, while genes in class 6 are responsible of the progression of senescence.

Even though senescence is viewed as a programmed process, many authors have distinguished it from apoptosis, a characteristic death program in animals cells which involves a selective death of groups of cells following a sequence of well definite steps (membrane blebbing, chromatin condensation and DNA fragmentation between nucleosomes being the most typical at the structural level), together with the activation of a number of genes, remarkably conserved during evolution (see Noodén *et al*. 1997, and references therein). Recent advances in the knowledge of events that induce the process of cell disorganization, as programmed cell death (PCD), have changed our appreciation of the development of senescence in plants (Danon *et al*. 2000; Thomas *et al*. 2003; Drury and Gallois 2006). Evidences of PCD have been shown in many aspects of plant development, as response to pathogens (Morel and Dangl 1997; Greenberg 1997), differentiation of tracheary elements (Fukuda 2000), cortical root aging (Liljeroth and Bryngelsson 2001), seed germination (Wang *et al*. 1998), flower aging and colouration/discoloration (reviewed in Teixeira da Silva 2006) and also leaf senescence (Yen and Yang 1998; Lee and Chen 2002). Despite the signal transduction pathways of many of these processes remain largely unknown, the involvement of reactive oxygen species (ROS) in the induction and/or progression of the signalling cascade seem to be a common factor as will be discussed in the next sections.

THE METABOLISM OF REACTIVE OXYGEN AND THE CONTROL OF SENESCENCE

Reactive oxygen species, such as hydrogen peroxide (H_2O_2), superoxide ion ($O_2^{\bullet -}$) and nitric oxide (NO) are well known by-products of energy-generating processes in chloroplasts and mitochondria. On the other hand, several extracellular and intracellular oxidases and peroxidases, including cell wall peroxidases and amine oxidases, enzymes from peroxisomes metabolism and plasma membrane-bound NADPH oxidases, also contribute to ROS production under different stress conditions (Frahry and Schopfer 1998; Corpas *et al*. 2001; Fath *et al*. 2002; Joo *et al*. 2005; van Breusegem and Dat 2006). At high concentrations, ROS behave as very reactive molecules, provoking serious alterations of protein structure, protein carbonilation, purine oxidations, DNA damage and lipid peroxidation of cellular and organellar membranes. Hence, a delicate regulation of ROS levels has to be maintained through a series of low molecule mass antioxidants (e.g. ascorbic acid, glutathione, tocopherols), enzymes regenerating the reducing forms of antioxidants, and ROS-interacting enzymes such as superoxide dismutases, peroxidases and catalases (Blokhina *et al*. 2003). While this tight homeostasis is necessary to prevent cellular damage, it also creates a baseline on which alterations in the level of specific ROS can act as endogenous signals in many physiological processes including cell elongation (Rodriguez *et al*. 2002); gene expression and enzyme activation (Guan and Scandalios 2000; Pastori and Foyer 2002; Yang and Poovaiah 2002); calcium signalling (Rentel and Knight 2004), and the initiation and/or propagation of stress responses to a wide variety of both biotic and abiotic factors (e.g. Corpas *et al*. 2001; Pastori and Foyer 2002; Kacperska 2004; Joo *et al*. 2005).

While the involvement of ROS in the control of PCD has been consistently demonstrated in animals, it was not until 1994, when Levine and co-workers showed that H_2O_2 orchestrates the hypersensitive disease resistance response in soybean cell suspensions, that there has been growing evidence that ROS, and particularly H_2O_2, are involved in the regulation of PCD in plants. In a recently published review, Gechev and Hille (2005) summarize the main lines

of evidence supporting the view that H_2O_2 is a key signal controlling several biological responses and developmental processes in plants, including PCD. Even though many questions still remain unanswered, the available information indicates that a wide range of endogenous and environmental signals can induce H_2O_2 accumulation, which in turns triggers the expression or repression of specific genes and transcription factors through alterations in Ca^{2+} fluxes, redox changes, activation of mitogen activated protein kinases (MAPK) cascades, and interaction with other signalling molecules like salicylic acid and NO.

LEAF SENESCENCE AND THE LIGHT ENVIRONMENT

Excess light and the induction of leaf senescence

The development of the senescence process is of essential importance in crop plants where the remobilization of nutrients such as nitrogen from the leaves, is the main source of nutrients for the developing organs or seeds.

In annual plants, in the absence of significant environmental stresses, reproduction is the main factor triggering leaf senescence. Nevertheless, other factors may initiate and/or modulate senescence rate both in the vegetative as well as the flowering stages. Among these factors, light has been shown to play an important role.

Under high irradiation conditions or continuous illumination, plants are in danger of absorbing more light energy than they can use for photosynthesis or other metabolic processes. When this occurs, an excess excitation energy (EEE) is generated (Mullineaux and Karpinski 2002). Dissipation of this EEE is usually achieved by a combination of photochemical and non-photochemical quenching processes. Among the former, the reduction of O_2 by electrons fed from the two photosystems together with increased rates of photorespiratory and chlororespiratory metabolism contribute to increase the level of ROS. As mentioned above, changes in ROS levels and in the redox state of photosynthetic electron transport components may in turn act as signals to activate further biochemical and physiological mechanisms to help plants acclimation to the light environment and maintain ROS homeostasis. However, a failure to do so leads to photo-oxidative damage and accelerated leaf senescence (e.g. Biswal 1995; Procházková and Wilhelmová 2004, and references therein).

Senescence induced by shading

In annual herbs and deciduous trees, the lower (older) leaves senesce earlier than the upper ones. While this has been frequently envisaged as an intrinsic process of leaf aging (e.g. Mooney *et al*. 1981), several lines of evidence suggest that changes in light quantity and/or quality across the canopy may act as signals to control leaf senescence. As for excess light, incubation of leaves in complete darkness also accelerates the development of senescence symptoms. This effect was shown to be associated to an enhancement of the activities of $O_2^{\bullet -}$- and H_2O_2-producing enzymes, a marked decrease of catalase (CAT) activity and a transition of peroxisomes into glyoxysomes (Pastori and del Río 1997, and references therein). In a study of the effect of darkness on either whole plants or individual leaves of *Arabidopsis* plants, Weaver and Amasino (2001) found that leaf senescence was not induced, but rather inhibited, when whole plants were placed in the dark, whereas it was strongly accelerated when individual leaves were darkened and the rest of the plant remained in the light. Interestingly, senescence rate of individually darkened leaves was higher in old as compared to younger leaves, suggesting that senescence may be induced not only in a localized fashion, but that darkness and age can act as separate and additive promoters of senescence. The fact that leaves senesced when individually darkened but not when the whole plant was darkened demonstrates that senescence of an individual leaf is controlled by the light status of the rest of the plant, although the mechanism responsible was not elucidated.

It has been suggested that a leaf can sense its light environment or photosynthetic status relative to those of other leaves in the plant by monitoring its sugar concentration, and increase, maintain or decrease its photosynthetic metabolism in order to maintain the distribution of key nutrients (particularly nitrogen) within the whole plant near the optimal conditions imposed by the environment (Ono *et al*. 2001). Several reports show that the induction of sugar accumulation in attached leaves as well as feeding detached leaves or protoplasts of leaf cells with glucose and/or sucrose cause decreases in the concentration of photosynthetic components and suppress the expression of photosynthetic genes (e.g. Harter *et al*. 1993; Ono *et al*. 2001; Wingler *et al*. 2006 and references therein). More recently it has been reported that glucose can upregulate the expression of *SAG 12*, a highly specific senescence-associated gene, in *Arabidopsis* plants (see Wingler *et al*. 2006), though the effect was observed only under low N nutrition. Hence it is possible that increments in the level of soluble carbohydrates in target leaves under particular illumination conditions can upregulate their senescence rate. To our knowledge the evidence supporting this hypothesis is mostly correlative, and data of other workers show that the effect of sugars on senescence traits and gene expression is quite complex and dependent on other factors (e.g. Thum *et al*. 2003; Wingler *et al*. 2006). Moreover, in antisense *BoINV2* (a gene for acid invertase) broccoli plants, postharvest floret senescence was delayed and cisteine protease mRNA transcription downregulated, despite soluble sugar content in the tissue significantly increased as compared to the wild type (Eason *et al*. 2007). On the other hand, despite the close relationship existing between ROS overproduction and the development of senescence symptoms, a role for carbohydrates in the control of the oxidative metabolism has not been clearly established.

In plant stands the decrease in light intensity is always accompanied by a depletion of wavelengths in the red and blue regions of the light spectra due to the presence of leaf pigments (mainly chlorophyll). It is well known that for plants the light environment is not only a source of energy but also of temporal and spatial information. As sessile and photoautotroph organisms, plants evolved a series of mechanisms to monitor the intensity, quality, direction and duration of light, and modulate their development in order to optimise energy acquisition and fitness (Whitelam and Devlin 1998; Smith 2000; Casal 2000; Casal *et al*. 2003). Plants sense the changes in the light environment through specific photoreceptors, which in turn trigger a wide number of physiological responses. Cryptochromes, phototropin and other yet unidentified photoreceptors are known to mediate responses to UV-A/B and blue light, while phytochromes mediate responses to changes in the red (R) and far-red (FR) light. Moreover, there is increasing evidence that green light can also elicit photomorphogenetic responses, though the photoreceptor/s involved have not been identified yet (Casal 2000; Spalding and Folta 2005 and references therein). Seed germination, seedling de-etiolation, chloroplast development and orientation, vegetative growth, shoot architecture and flowering are some of the processes regulated by light signals. The mode of action of plant photoreceptors has been a matter of intensive research (see reviews by Smith 2000, Casal 2000; Fankhauser 2002; Casal *et al*. 2003; Spalding and Folta 2005) and will not be discussed in detail in the present review. Basically, conformational changes of the photoreceptor molecule induced by light lead to at least two separate mechanisms of action: one that results in the selective expression/repression of target genes (many of which are trasnscription factors) and another that rapidly and reversibly operates to modulate cellular ionic balance. Ubiquitin-mediated proteolisis of either the photoreceptor protein or target proteins is also one of the prominent components of phytochrome and cryptochrome signal transduction pathways (Spalding and Folta 2005). Despite there has

been a considerable advance in the knowledge of the mode of action of the different photoreceptors in the above mentioned processes, their role in the regulation of leaf senescence remains still largely unknown.

In certain plant species, leaf senescence (as measured by chlorophyll and/or protein degradation rates) is accelerated when leaves are exposed to FR enriched light, and this effect may be suppressed by application of R light (Biswal and Biswal 1984; Guiamét *et al*. 1989; Rousseaux *et al*. 1996). On the other hand, the senescence symptoms induced by illuminating leaf spots of tobacco plants with FR light were overcame in tobacco transformants overexpressing an oat phytochrome cDNA (Rousseaux *et al*. 1997). While these data strongly suggest that phytochromes might play a key role in the perception of light signals inducing senescence in shaded leaves of dense stands, the fact that shading with black or neutral light filters can mimic the effects of natural shade in either wild type or phytochrome mutants (e.g. Smith *et al*. 1993; Weaver and Amasino 2001) indicates that other factors are involved. For example, Quiles Rodenas and co-workers (1988) reported that in barley plants, the active forms of phytochrome and a near ultraviolet light receptor have independent contributions to the retardation of leaf senescence. In a recent publication we showed that a decrease in light intensity and/or a reduction in the R/FR ratio were not sufficient conditions to induce senescence symptoms in excised wheat leaves exposed to selective light filters. Rather, soluble protein and chlorophyll degradation rates (Causin *et al*. 2006b) as well as endopeptidase activity (Causin *et al*. 2006a) consistently increased when light transmission between 350 and 450 nm was markedly suppressed. As shown in **Fig. 1**, a similar effect can be observed on intact leaves, indicating that leaf excision accelerated but did not alter the senescence pattern caused by the different light treatments. In our experimental conditions, the retardation of leaf senescence when blue light was not suppressed was correlated to a decrease in lipid peroxidation and a higher activity of tissue CAT, an enzyme which has been shown to play a key role in the prevention of oxidative damage and the development of senescence symptoms under different environmental stresses (e.g. Pastori and del Río 1997; Willekens *et al*. 1997; Vandenabeele *et al*. 2004). Moreover, infiltration of leaves with a solution of CAT from bovine liver retarded the development of senescence symptoms in the absence of blue light (Causin *et al*. 2006a). Altogether these data suggest that the deprivation of specific wavelengths in shaded leaves might affect leaf senescence rate by modulating some key components of the oxidative metabolism. Our results also indicate that the effect of blue light on the retardation of leaf senescence probably involves calcium signalling as well as the action of cytokinins (see Causin *et al*. 2006a, 2006b), although the precise mechanism remains to be elucidated.

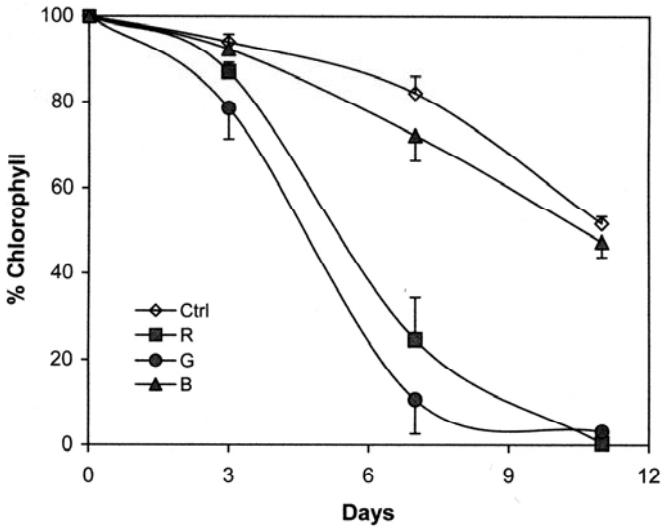

Fig. 1 Changes in chlorophyll concentration (expressed as % of initial concentration) in intact wheat leaves exposed to either transparent acetate (control, R/FR= 1.01) or different Lee filters (red #026, R/FR= 0.86; green #089, R/FR= 0.12; or blue #075, R/FR= 0.13) during 11 days. Wheat plants were grown in a greenhouse under natural photoperiod as described in Causin *et al*. (2006b). Maximum transmitted photosynthetic active radiation (PAR), as measured with a Li-190 quantum sensor (LI-COR, Lincoln, USA) attached to a CAVA-RAD data logger (Cavadevices, Argentina), did not significantly differ among the Lee filters. Twenty seven days after sowing, the middle portion of the 4th leaf of randomly chosen individuals was covered with a 1.7 cm x 10.0 cm (diameter x length) cylinder made with the above mentioned filters, and kept horizontal during the whole experimental period. Total chlorophyll concentration at the indicated time intervals was measured with a SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Japan) and estimated against a standard curve according to Porra, 2002. Data are mean of four independent replicates ± SE.

CONCLUDING REMARKS

Light is a source of either energy as well as environmental information for all green plants, and thus their individual modules (leaves) should adapt their metabolism and life span in order to optimize whole-plant carbon gain per unit N together with plant competitive ability for light. For example, in transgenic tobacco plants with delayed leaf senescence it was shown that the carbon gain in these plants would increase if the shed lower leaves senesced and reallocated the N to form more leaf area (Boonman *et al*. 2006). Among the factors controlling leaf senescence, signals derived form the light environment may play an important role.

Except for the involvement of specific photoreceptors, little is known about the biochemistry of leaf senescence induced by changes in the light environment. The evidence summarized in the present review support the idea that, as for other biotic and abiotic factors, the effect would be exerted through changes in ROS metabolism. In fact, literature data show that excess illumination as well as darkening of individual leaves can trigger leaf senescence, and that in both cases this phenomenon is associated to an increase in the production of ROS beyond homeostatic levels. Similarly, it was shown that the senescence symptoms induced by shading in wheat leaves are correlated to a down-regulation of the antioxidant enzyme CAT when specific wavelengths are deprived. A failure to avoid or dissipate accumulating EEE within the chloroplast seems to be the primary cause of ROS overproduction when the light energy encountered by the leaf is in excess of that needed to attain its maximum photosynthetic productivity. On the contrary, the physiological mechanisms leading to ROS accumulation in shaded leaves are less understood, though the experimental evidence indicates that photoreceptor- and/or sugar-mediated signalling pathways may be involved.

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