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REVIEW

Leaf senescence and activities of the antioxidant enzymes

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Abstract

Senescence is a genetically regulated process that involves decomposition of cellular structures and distribution of the products of this degradation to other plant parts. Reactions involving reactive oxygen species are the intrinsic features of these processes and their role in senescence is suggested. The malfunction of protection against destruction induced by reactive oxygen species could be the starting point of senescence. This article reviews biochemical changes during senescence in relation to reactive oxygen species and changes in antioxidant protection.

Additional key words: ageing, enzymatic antioxidants, lipid peroxidation, non-enzymatic antioxidants, oxidative stress.

Introduction

Leaf senescence – a final developmental phase between maturity and death of a plant or a plant part - has been defined as a highly organized, energy-requiring, genetically programmed process.

The nature of the inducer(s), initiating a natural senescence program, has not been fully explained yet. Among the various possibilities, such as source-sink competitions during redirection of nutrients, changing hormonal control, or changes of the energy status of the cell (Thimann 1980, Thomas and Stoddart 1980, Kar and Feierabend 1984, Buchanan-Wollaston 1997), the free-radical theory has attracted attention. A hypothesis has been developed that ageing results from an accumulation of harmful free radicals (Harman 1981) and that the onset of senescence is mainly due to the uncontrolled strong enhancement in the generation of reactive oxygen species (ROS), especially superoxide, singlet oxygen, hydroxyl radical and hydrogen peroxide (Thompson and Barber 1987).

Three phases may be distinguished in a typical senescent process (Peñarrubia and Moreno 1995):

1) Storage mobilization. A phase of selective degradation of certain molecules. This degradation does not cause a major impairment of the physiological functions. The mobilized molecules may be considered as nutrient storage materials. In some cases senescence may be reversed during this phase by certain changes in environmental conditions.

2) Generalized breakdown. Extension and generalization of breakdown to components which are central in maintaining physiological function. As a result of this breakdown a physiological function is consequently lost. During this phase the senescence process becomes irreversible and death of the cells becomes inevitable.

3) Abscission. The final phase of senescence is abscission and death.

Leaf senescence is accompanied by changes of many organelles. Chloroplasts of senescing leaves show reduced volume, their shape is spherical and the thylakoid system is reduced (Matile 1992). The final developmental stage of chloroplasts – gerontoplasts – shows an increase in the number and diameter of plastoglobuli, a reduction of the thylakoid system, a loosening of the stacking of thylakoids and a swelling of intrathylakoid spaces. Mitochondria remain intact until late senescence, however, in later stages some swelling or distortion of cristae becomes apparent. Nuclei do not show substantial structural changes until relatively late stage of senescence

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Abbreviations: APX - ascorbate peroxidase; Asc - ascorbate; CAT - catalase; Chl - chlorophyll; DHA - dehydroascorbate; DHAR - dehydroascorbate reductase; GPX - glutathione peroxidase; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; MDHA - monodehydroascorbate radical; MDHAR - monodehydroascorbate reductase; ROS - reactive oxygen species; SOD - superoxide dismutase; α-toc - α-tocopherol; β-car - β-carotene.

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(Noodén *et al*. 1997), nevertheless, there are some reports of chromatin condensation and other changes (Kuran 1993, Biradar and Rayburn 1994, Noodén *et al.* 1997).

Biochemical changes during senescence

Lipids: One of the most characteristic features of senescence is the initiation of membrane leakiness and a consequent loss of intracellular compartmentalization predominantly due to lipid damage. Lipid peroxidation is commonly used as an indicator of prevalence of free radicals in tissues (Smirnoff 1993). Lipid peroxidation not only threatens the integrity and function of membranes and membranous proteins but also produces a variety of toxic aldehydes and ketones (Valentine *et al*. 1998, Wilhelmová *et al*. 2006). Some of such products of lipid peroxidation, malondialdehyde and 4-hydroxy-nonenal, cause protein damage by means of reactions with lysine amino groups, cysteine sulfhydryl groups and histidine imidazole groups (Esterbauer *et al*. 1991, Uchida and Stadtman 1992, Friguet *et al*. 1994, Brunner *et al*. 1995, Refsgaard *et al*. 2000).

The striking chemical change in the membrane lipids is a dramatic increase in the sterol/phospholipid ratio (Borochov *et al*. 1978, McKersie *et al*. 1978, Lees and Thompson 1980), however, content of sterols declines with physiological ageing (Paliyath and Droillard 1992).

Photosynthetic pigments: Breakdown of chlorophylls (Chl) may be one of the earliest symptoms of senescence. All final catabolites are not derived only from chlorophyll *a* (Chl *a*) but represent the breakdown products of chlorophyll *b* (Chl *b*) as well (Curty and Engel 1996, Ginsburg and Matile 1993, Matile and Hörtensteiner 1999). The first step in the degradation of Chl *a* is hydrolysis of the phytyl ester bond catalyzed by chlorophyllase, which forms chlorophyllide *a* and phytol (Tsuchiya *et al.* 1997, Suzuki and Shioi 1999). In the next step magnesium is released from the macrocyclic ring and pheophorbide *a* forms with the action of Mg-dechelatase substance (Shioi *et al*. 1996a, Suzuki and Shioi 1999). The final step of macrocycle ring modification is the conversion of pheophorbide *a* to pyropheophorbide *a* (Shioi *et al*. 1996b, Watanabe *et al.* 1999, Suzuki and Shioi 1999). The next reaction, oxidative cleavage of the tetrapyrrole ring, is probably catalyzed by pheophorbide *a* oxygenase (Matile *et al.* 1996, Rodoni *et al.* 1997, Suzuki and Shioi 1999).

The main catabolites of degraded chlorophylls are derivatives of bilin (Engel *et al.* 1991, Iturraspe and Moyano 1995, Doi *et al.* 1997, Suzuki and Shioi 1999). The final products have been also termed nonfluorescent Chl catabolites (Matile and Hörtensteiner 1999). These linear tetrapyrroles are accumulated in the vacuoles of senescent mesophyll cells (Matile *et al.* 1988, Kräutler 2003, Hörtensteiner 2004).

Nucleic acids: The content of DNA remains relatively constant during senescence (Makrides and Goldthwaite 1981, Buchanan-Wollaston 1997). It has been shown that repeated sequences are selectively degraded while coding regions of nuclear DNA remain to a large extent intact (Abeles and Dunn 1990).

Total RNA content decreases during senescence, with a completely yellow leaf having about 10-times less RNA than a green one (Lohman *et al.* 1994). The expression of many genes is switched off (Bate *et al.* 1991, Hensel *et al.* 1993, Lohman *et al.* 1994). New transcripts were originally detected as a changed pattern of products observed in *in vitro* translation experiments using mRNA from senescing leaves when compared to green leaves (Malik 1987, Thomas *et al.* 1992).

Transfer RNA synthetase activities are greatly reduced during senescence (Jaybaskaran *et al.* 1990). A general increase in RNase activities has been described during senescence (Green 1994, Buchanan-Wollaston 1997). Nevertheless, qualitative changes in mRNA are probably more relevant in the senescence process.

The fate of the purines and pyrimidines resulting from nucleic acid degradation is not clear, but they are presumably broken down further, to keep their nitrogen and carbon components for further use in the plant. The increased levels of uricase and xanthine oxidase observed in the peroxisomes of senescent leaves indicate that purine catabolism takes place in this organelle (Vicentini and Matile 1993, Pastori and del Río 1994).

The nucleic acids, in particular the rRNA, are used as an important source of carbon, nitrogen and especially phosphorus within a senescing cell.

Proteins: Leaf senescence is characterized by a progressive decrease of total protein content (Brady 1988). The patterns of protein loss are characteristic and independent of the cause of senescence. Many specific proteins are degraded while others remain intact.

Probably both reduced synthesis and enhanced proteolysis are responsible for protein loss observed during senescence. In this regard, synthesis of all thylakoid proteins is known to be severely curtailed in senescing bean leaves except for the D1 protein of photosystem 2 (Droillard *et al.* 1992). Increased protein breakdown may result from different mechanisms: *de novo* synthesis of proteolytic enzymes, activation of pre-existing proteases, decompartmentalization of proteases and their substrates for degradation.

Reaction of most ROS with proteins results in the initial formation of carbon-centered radicals at either the protein backbone or side chains (Hawkins and Davies 2001).

Oxidation of the protein backbone: The carbon-centered radical reacts rapidly with O_2 to form an

alkylperoxyl radical intermediate, which can give rise to the alkylperoxide, followed by a formation of an alkoxyl radical, which may be converted to a hydroxyl protein derivate. The alkyl, alkylperoxyl and alkoxyl radical intermediates in this pathway may undergo side reactions with other amino acid residues in the same or a different protein molecule to generate a new carbon-centered radical (Berlett and Stadtman 1997).

Oxidation of amino acid side chains: Carbon-centered radicals react rapidly with O_2 to give peroxyl species. Peroxyl radicals undergo a number of reactions that result in the formation of carbonyl groups (aldehydes or ketones), alcohols and hydroperoxides. Alkoxyl radicals can be generated from peroxyl radicals. They can undergo rapid addition and hydrogen abstraction reactions, as well as facile unimolecular fragmentation and rearrangement reactions (Hawkins and Davies 2001).

Protection against reactive oxygen species during senescence

ROS are accumulated with age in a progressive manner. Their most powerful source is chloroplast (Foyer and Noctor 2000). Other sources are electron transport chain in mitochondria, oxidation in microbodies (Dat *et al.* 2000) or cell wall peroxidises (Elstner 1982). Plants have evolved sophisticated mechanisms to maintain ROS concentrations under the control (see Table 1).

Leaf senescence is often associated with increased oxidative damage to cellular macromolecules by ROS. Since ROS levels and their damage products in many plants are known to increase during senescence, it is possible that these changes are due to a decline in the activity of certain antioxidant enzymes. There is superiority of evidence from number of laboratories that antioxidant protection declines at the later stages of leaf senescence. However, it is still impossible to draw any explicit conclusion. For example, Scebba *et al.* (2001) described entirely different courses of the activities on two different cultivars of *Prunus armeniaca* during senescence. Another illustration of a surprising course of antioxidant activity was described in *Arabidopsis* where APX activity dramatically decreased after pre-bolt stage and the lowest was in medium bolting stage. Its activity rebounded with further development (reproduction and seed setting) and this increase precedes the onset of visible senescence symptoms (Ye *et al.* 2000).

Table 1. Compendium of the most important enzymatic and non-enzymatic antioxidants.

Antioxidant	Reaction
Superoxide dismutase (SOD)	$2\,O_2^{\cdot-} + 2\,H^+ \Rightarrow O_2 + H_2O_2$
Ascorbate peroxidase (APX)	$H_2O_2 + 2\,\text{Asc} \Rightarrow 2\,H_2O + 2\,\text{MDHA}$
Monodehydroascorbate reductase (MDHAR)	$2\,\text{MDHA} + \text{NAD(P)H} + H^+ \Rightarrow 2\,\text{Asc} + \text{NAD(P)}^+$
Dehydroascorbate reductase (DHAR)	$\text{DHA} + 2\,\text{GSH} \Rightarrow \text{Asc} + \text{GSSG}$
Glutathione reductase (GR)	$\text{GSSG} + \text{NAD(P)H} + H^+ \Rightarrow 2\,\text{GSH} + \text{NAD(P)}^+$
Catalase (CAT)	$2\,H_2O_2 \Rightarrow 2\,H_2O + O_2$
Glutathione peroxidase (GPX)	$H_2O_2 + 2\,\text{GSH} \Rightarrow 2\,H_2O + \text{GSSG}$
Glutathione (GSH)	$H_2O_2 + 2\,\text{GSH} \Rightarrow 2\,H_2O + \text{GSSG}$
	$\text{GSH} + OH^{\cdot} \Rightarrow H_2O + \text{GS}^{\cdot}$
Ascorbate (Asc)	$\text{Asc} + H_2O_2 \Rightarrow 2\,H_2O + \text{DHA}$
β-carotene (β-car)	$\beta\text{-car} + ROO^{\cdot} \Rightarrow \beta\text{-car}^{\cdot} + ROOH$
	$^3\text{Chl}^* + \beta\text{-car} \Rightarrow {}^1\text{Chl} + {}^3\beta\text{-car}^*$
α-tocopherol (α-toc)	$\alpha\text{-toc} + LOO^{\cdot} \Rightarrow \alpha\text{-toc}^{\cdot} + LOOH$

The course of activity in various experiments depends on too many factors: time of sampling, length of monitored time course, a kind of a method employed, plant organ, plant species or cultivar. A selection of reference unit is very important. Almost all of them undergo quantitative change to various extents. The tendency of studied characteristics can achieve even contradictory course on dependence of such reference parameter (per fresh mass, dry mass, proteins, per volume of enzyme extract or per one leaf, one cotyledon, *etc*.).

The antioxidant activity in senescence varies on the level of organelles as well. For example a notable increase in the activity of peroxisomal MnSOD and a decline in the activity of mitochondrial MnSOD from pea leaf extract were determined at the same time (Pastori and del Río 1994). On the other hand a decrease in the activity of whole pea leaf extract MnSOD was described in another experiment (Jimenéz *et al.* 1998). Moreover differences between natural and artificially induced senescence would deserve a separate article.

Table 2. The antioxidant enzyme activity and content of non-enzymatic antioxidant during plant senescence. Symbol ↑ describes continuous increase of activity or concentration during monitored time course; symbol ↕ describes initiative increase, followed by a decrease of activity or concentration; symbol ↓ describes continuous decline of activity or concentration during monitored time course.

Antioxidant	Plant	Organ	Course	Reference
Total SOD	clusterbean	cotyledons	↓	Deo *et al.* 2006
	tobacco	leaves	↕	Dertinger *et al.* 2003
	bean	cotyledons	↕	Procházková and Wilhelmová 2007a
	maize	leaves	↕	Procházková *et al.* 2001
	wheat	leaves	↓	Srivalli and Khanna-Chopra 2001
CuZnSOD	bean	cotyledons	↕	Procházková and Wilhelmová 2007a
MnSOD	bean	cotyledons	↕	Procházková and Wilhelmová 2007a
	pea	leaves	↓	Jimenéz *et al.* 1998
APX	pea	leaves	↓	Jimenéz *et al.* 1998
	cucumber	cotyledons	↑	Kanazawa *et al.* 2000
	bean	cotyledons	↕	Procházková and Wilhelmová 2004
	maize	leaves	↕	Procházková *et al.* 2001
CAT	cucumber	cotyledons	↓	Kanazawa *et al.* 2000
	soybean	leaves	↕	Fu *et al.* 2000
	maize	leaves	↕	Procházková *et al.* 2001
	bean	cotyledons	↓	Procházková and Wilhelmová 2004
	Arabidopsis	leaves	↓	Zimmermann *et al.* 2006
GR	cucumber	cotyledons	↕	Kanazawa *et al.* 2000
	bean	cotyledons	↕	Procházková and Wilhelmová 2004
	tobacco	leaves	↓	Dertinger *et al.* 2003
DHAR	cucumber	cotyledons	↓	Kanazawa *et al.* 2000
GPX	cucumber	cotyledons	↑	Kanazawa *et al.* 2000
GSH	bean	cotyledons	↓	Procházková and Wilhelmová 2007b
α-toc	sage	leaves	↓	Munné-Bosch *et al.* 2001
	tobacco	leaves	↑	Dertinger *et al.* 2003
Asc	tobacco	leaves	↓	Dertinger *et al.* 2003
	bean	cotyledons	↓	Procházková and Wilhelmová 2007b

Conclusion

Recent studies unravelled many questions concerning senescence and ROS, but many other questions arise. The focus of the current research is aimed rather at increasing our knowledge of leaf senescence at the molecular level. The identification and the characterization of genes involved in the complex degradation events that occur during leaf senescence and the elucidation of their mechanisms of regulation will help in increasing of yield, stress resistance and shelf life of agricultural products.

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