

Senescence in Plants

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Senescence is the final stage of plant development during which the plant reclaims the valuable cellular building blocks that have been deposited in the leaves and other parts of the plant during growth. Maintaining an efficient senescence process is essential for survival of the plant or its future generations. Senescence is a complex highly regulated process that requires new gene expression and involves the interactions of many signalling pathways.

Introduction

Senescence in plants is a complex, developmentally regulated phase during which cellular structures are carefully dismantled and the soluble components transferred to other parts of the plant, either for storage or to promote further growth. All parts of the plant undergo senescence but for the purpose of this article the emphasis will mainly be on the senescence of leaves since this has been the most studied in recent years.

Leaf senescence occurs when the leaf is no longer of use to the plant. This may be due to the stage of development of the plant, the age of the leaf or be induced by environmental factors. As plants enter the flowering and seed development stage, the nutrients within the leaves are required for the seed development and so, in this case, senescence is induced by developmental signals. Alternatively, plant growth results in the leaves at the base of the plant becoming shaded and restricted in their photosynthetic potential, in this case senescence is induced by environmentally controlled signals. Other types of environmental stress such as water and nutrient limitation or pathogen attack can also affect plant growth and bring about premature senescence. Senescence is at its most obvious in the autumn when the dramatic colours of deciduous trees are striking reminder of the changing seasons (**Figure 1a**). This senescence is caused by seasonal changes such as light and temperature. A detailed molecular study in poplar in Sweden has indicated that senescence starts on exactly the same day each year irrespective of environmental condition and follows the same degradative pathways. This implies that, for this species, photoperiod is the sole regulator causing the onset of senescence.

In perennial plants and trees, the mobilized components are stored in roots or trunks to provide the building blocks for growth in the following year. In annual plants, such as wheat, soybean and the model plant species, *Arabidopsis*, the entire plant undergoes senescence and all reusable nutrients are stored in the seed (**Figure 1b**).

Introductory article

Article Contents

- Introduction
- Changes that Occur during Senescence
- Gene Expression during Senescence
- Regulation of Leaf Senescence
- Value of Senescence Research

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Changes that Occur during Senescence

Once a leaf is destined for senescence, it enters a highly regulated programmed series of events by which its cellular components are dismantled, degraded and mobilized. In the poplar analysis described above, it was shown that over 80% of the nitrogen and phosphorous were reclaimed from the senescing leaves. This process is controlled and usually the leaf is maintained in a viable state until the remobilization is complete. The high degree of control is shown clearly by the fact that senescence can be reversed, if the process has not proceeded beyond a certain stage. Chloroplasts can be induced to regreen and to start photosynthesizing again.

Loss of chlorophyll, with the resulting yellowing of the leaves is the most obvious sign of senescence and in some cases, as seen in **Figure 1a** and **c**, the additional synthesis of anthocyanins during senescence results in dramatic orange, red and even purple coloration. Synthesis of anthocyanins by certain species has been shown to be important to protect the leaf from photooxidative damage and maintain viability as senescence progresses. Another noticeable fact of leaf senescence is illustrated in the leaves shown in **Figure 1c**. Often the leaf does not senesce evenly; the tip and edges usually show the first signs with the areas around the vein tissues and the end of the leaf nearest the plant being the last to senesce. The reason for this is obvious if it is considered that the purpose of senescence is to remove soluble components from the leaf, via the vein system, before the leaf is allowed to die and drop off the plant. Cells around the veins are kept active until maximum mobilization has occurred.

Structural changes

The majority of the protein and lipid component of a leaf is in the chloroplast and this is where the first and most



Figure 1 Illustrations of plant senescence: (a) Autumnal senescence in a beech wood, Derbyshire. (b) Total senescence in the entire wheat plant results in all mobilizable nutrients being stored in the grain. (c) Leaf senescence showing differential progression of senescence in the leaf. Areas close to the veins senesce last.

obvious structural changes occur during senescence. The highly organized thylakoid membrane stacks, characteristic of chloroplasts in mature green leaves become dispersed and by mid-senescence, the membrane structure has become totally disorganized. The formation of large electron dense globules called plastoglobuli is characteristic of the senescence of chloroplasts. These bodies are thought to have a role in lipid mobilization but may also be involved in the degradation of chlorophyll. Mitochondria provide essential energy to maintain the protein synthesis and enzyme activity required for senescence to progress and these organelles are maintained until the final stages. As senescence progresses, the plasma and vacuolar membranes finally become disrupted and cell death occurs.

Chlorophyll degradation

Leaf yellowing is the first visible sign of senescence but chlorophyll levels have started to drop well before yellowing is clearly visible. **Figure 2** shows the changes in chlorophyll levels in the same *Arabidopsis* leaf harvested at different time points during development and it is clear that 50% of chlorophyll is lost before there are obvious signs of yellowing in the leaf. In mature green leaf cells, chlorophyll is bound to apoproteins in the thylakoid and this complex is responsible for light trapping during photosynthesis. During senescence, the N content of these apoproteins (which comprises around 30% of the total plastid nitrogen) must be remobilized, and chlorophyll is released. The first steps of chlorophyll degradation are common to all plants and involve several

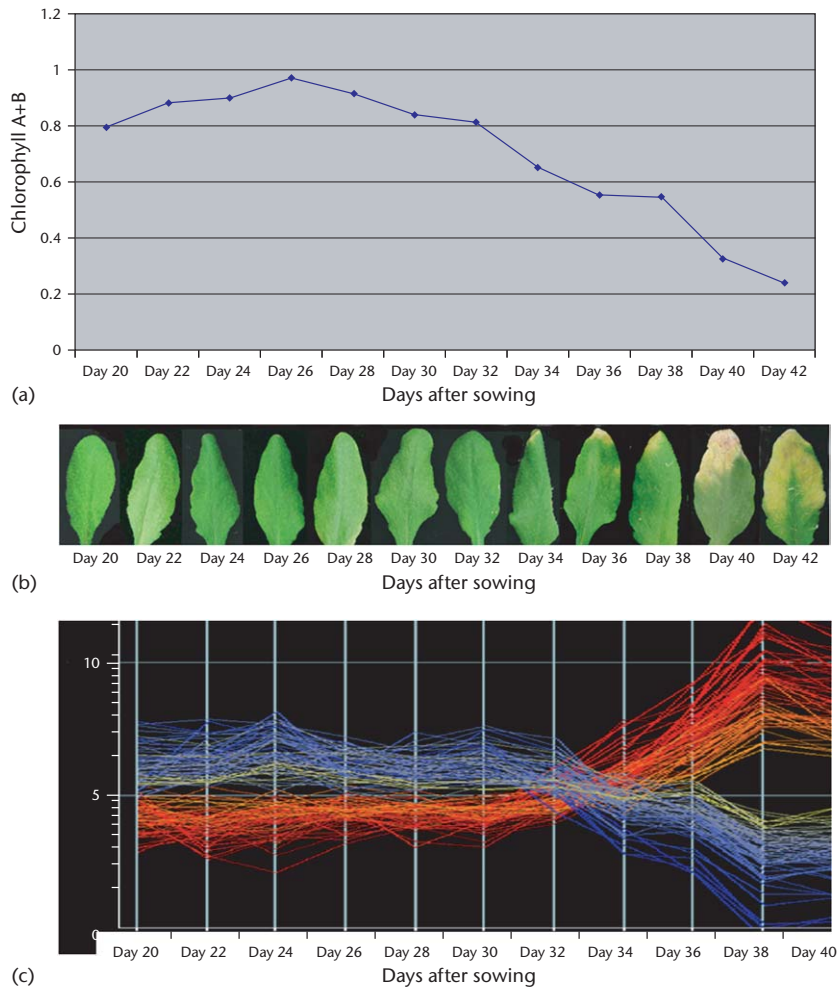


Figure 2 Changes in the *Arabidopsis* leaf during development. (a) Change in chlorophyll levels as senescence progresses (b) Change in visual appearance of leaf 7 harvested from *Arabidopsis* Col0 plants at different time points during development (c) Gene expression changes during leaf development. GeneSpring (Silicon Genetics) analysis shows two clusters of genes each line shows the changes in expression of a single gene, those in red show increased expression during development while those in blue show a decrease. SAG12 is one of the genes in the red cluster, several chlorophyll binding proteins and other photosynthetic proteins are in the blue cluster.

enzymatic steps leading to the production of colourless compounds (primary fluorescent chlorophyll catabolites, pFCCs). Following this the processes are different in different species, pFCCs undergo various modifications before being transported from the chloroplast and stored in the vacuole as nonfluorescent chlorophyll catabolites (NCCs). Chlorophyll degradation starts with the disruption of the thylakoid structure allowing access to the proteins that bind chlorophyll in the membrane. Chlorophyllase catalyses the conversion of chlorophyll into chlorophyllide which is then converted into pheophorbide *a*, followed by red chlorophyll catabolite (RCC) and then to the pFCC intermediate. The genes involved in the pathway for chlorophyll degradation in plants have been identified in the last few years. The cloning of the genes encoding the three key enzymes chlorophyllase, pheophorbide aoxigenase (PAO) and red

chlorophyll catabolite reductase (RCCR) has been an essential step in the elucidation of the degradation pathway.

Once chlorophyll is separated from the proteins that bind it in the chloroplast membranes it becomes highly toxic, being very reactive to light. Therefore it is very important that chlorophyll is rapidly degraded as a protective measure against phototoxicity. Interestingly, two of the key enzymes in the chlorophyll degradation pathway (POA and RCCR) were identified originally in *Arabidopsis* mutants showing a light-dependent cell-death phenotype. In the absence of the complete chlorophyll-degradation pathway, the build up of toxic intermediates results in cell death and plants lacking these enzymes are extremely vulnerable to light stress. Therefore having a functional chlorophyll degradation pathway is vitally important for plant development and survival.

Protein degradation

Proteins are degraded during leaf senescence to release valuable nitrogen and other minerals for mobilization. The level of total protein falls rapidly during leaf senescence, and this is paralleled by an increase in transcription and activity of a number of different types of protease including vacuolar processing enzymes. The majority of leaf protein is stored in the chloroplast but, perplexingly, the majority of the proteolytic activity associated with senescing leaves appears to be in the vacuole. Recently, the importance of autophagy in the senescence process has become evident with the identification of several senescence-enhanced genes in *Arabidopsis* encoding orthologues of yeast autophagic genes. Autophagy is a common eukaryotic process by which cytoplasm and organelles are taken up by the vacuole for degradation. *Arabidopsis* mutants in two autophagy genes show an accelerated senescence phenotype indicating that regulated autophagic activity is essential for controlled senescence. In addition, the occurrence of different types of vacuole has been observed in senescing leaf; a population of small lytic vacuoles develop in the peripheral cytoplasm, close to the chloroplasts. These small vacuoles have been shown to contain the senescence-specific protease SAG12, and are likely to have an important role in protein degradation during senescence.

The amino acids released by protein degradation are mobilized from the leaf and transported in the phloem. There is some conversion of amino acids into glutamine and asparagine, the levels of these are disproportionately high in the phloem leaving a senescing leaf and both glutamine and asparagine synthetase transcript levels and activity increase during leaf senescence. Conversion into glutamine or asparagine is efficient since this allows the transport of extra N moieties per molecule.

Other macromolecules

Lipids are remobilized during senescence via a process of β -oxidation and gluconeogenesis. Some carbon may be mobilized from a senescing leaf but it is likely that most of the lipid is used to provide energy for the other senescence processes to occur since the level of energy supplied by photosynthesis is obviously reduced as senescence progresses. Nucleic acids, especially RNA, provide a valuable source of mobilizable phosphorous and RNA levels drop rapidly as senescence enters the final stages. The nuclear DNA levels remain stable to the final stages of senescence to enable *de novo* transcription capability to be maintained.

Gene Expression during Senescence

Some time ago, it was shown that senescence is an active process that requires new genes to be transcribed and proteins to be synthesized. Since then, using a variety of molecular techniques culminating in microarray analysis,

hundreds of senescence-enhanced genes have been identified and these are implicated as encoding proteins that have a role in the senescence process. However, the actual function for the vast majority of these genes remains a mystery. Microarray experiments using *Arabidopsis* gene probes and RNA isolated from green and senescent *Arabidopsis* leaves have shown that at least 800 genes are increased in expression during senescence and approximately the same number are downregulated. The functions of some of these genes can be inferred from their annotation. **Figure 3** illustrates the gene ontology (GO) annotations (<http://www.geneontology.org>) for the groups of up and downregulated genes and shows that many more regulatory factors are upregulated including kinases, transcription factors and protein binding factors. This illustrates the considerable regulatory activity that is an essential feature of the senescence process. For example, amongst the upregulated genes there are around 80 putative transcription factors. In addition, there are many genes encoding degradative functions, proteases, nucleases, cell wall and lipid degradation enzymes, etc. Unsurprisingly, a major group of the downregulated genes encode proteins involved in chloroplast development, chlorophyll biosynthesis and photosynthetic processes. A further experiment recently undertaken in the authors lab, using RNA isolated from 11 time points during leaf development has shown that there are multiple patterns of gene expression within this collection with groups of genes showing different times of expression. **Figure 2** illustrates two groups of genes showing opposite expression patterns; the protease encoding gene SAG12 is in the group of upregulated genes while many genes involved in photosynthesis are in the group of downregulated genes. It is likely that genes involved in the same degradative pathway will be co-regulated and the mechanisms by which expression of such gene clusters is controlled are currently being investigated with the long-term aim of identifying the key regulators of senescence.

Senescence can be induced artificially by dark treatment or by removal of the leaf from the plant. These treatments cause yellowing and protein degradation but are not necessarily true representation of the natural process of developmental senescence. Recent microarray experiments comparing developmental senescence with artificially induced senescence have indicated many common features but also some significant differences. For example, the signalling pathway involving the hormone salicylic acid is important in developmental senescence but is not activated in artificially induced senescence.

Regulation of Leaf Senescence

Hormonal regulation

Many plant hormones have been implicated as having a role in controlling leaf senescence and different plant species may have different hormone dependencies. In general, cytokinin

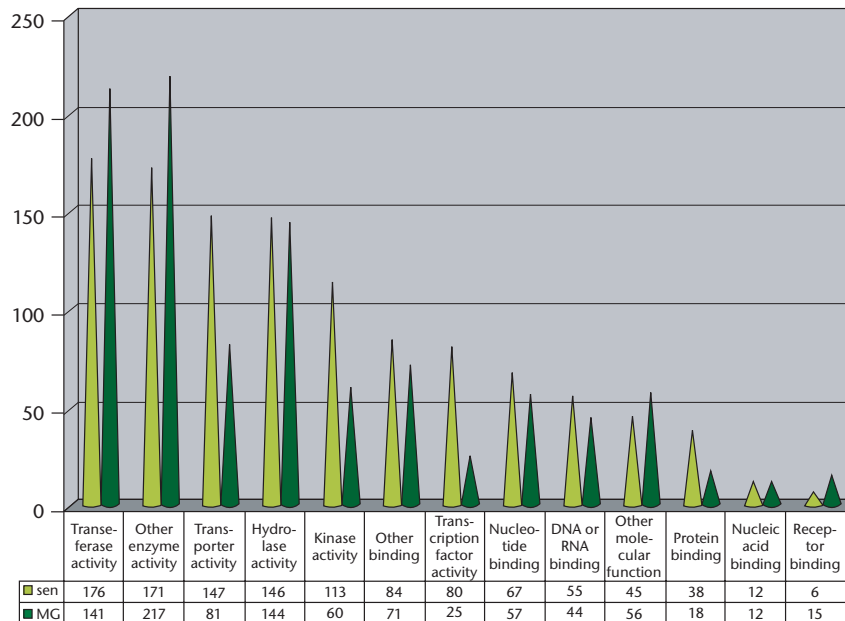


Figure 3 Potential functions of genes up or downregulated during senescence. Gene Ontology (GO) annotations were applied to the groups of genes that were either up or downregulated during senescence. Senescence enhanced genes (sen) are in pale green, while genes downregulated in senescence (MG) are shown in dark green. Functional classifications are shown under each pair of cones and the numbers of genes in each group are shown in the table below.

is seen as being a negative regulator of senescence since treatment of leaves with cytokinin delays the onset of senescence. An elegant experiment using transgenic tobacco expressing a cytokinin biosynthesis gene from a senescence-enhanced promoter showed the importance of cytokinin very clearly. At the onset of senescence, the promoter was induced and the synthesis of cytokinin occurred. This resulted in inhibition of senescence and the leaves remained green.

Hormones with a potential role in the induction of senescence include ethylene, salicylic acid and jasmonic acid. Levels of all these hormones increase during senescence and different groups of senescence-enhanced genes depend on each pathway for expression. However, the role and importance of each in the senescence process is not always clear. Mutants in ethylene signalling show delayed leaf senescence but unlike fruit that depend on ethylene for ripening, leaves lacking the ethylene signal do senesce eventually. Ethylene signal perception is age dependent in *Arabidopsis*; young leaves treated with ethylene are not induced to senesce, but once a certain age threshold has been reached, ethylene treatment results in the rapid induction of early senescence. Treatment of *Arabidopsis* leaves with jasmonic acid (JA) results in the induction of senescence but mutants in JA signalling do not show an obvious delayed senescence phenotype. All these three hormones have strong links to stress signalling and the importance of them in controlling gene expression during senescence is indicative of the considerable cross talk that exists between senescence and stress response pathways.

Regulatory genes

Several potential regulatory genes have been identified by map-based cloning from *Arabidopsis* mutants showing delayed leaf senescence. These include mutants in genes controlling the signalling pathways described above and also a small number of genes with unknown functions. For example, the *ore9* gene encodes a protein with a potential role in protein degradation, *ore4* encodes a plastid ribosomal subunit, etc. Recently, a gene encoding a NAC domain transcription factor has been shown to be important for normal senescence. However, in all these mutants senescence is delayed but not stopped; leaves do eventually enter what appears to be a normal senescence process. There is no information yet on what the role of these genes is in the senescence process and no mutants have been identified that inhibit senescence totally. The reason for this is probably due to the plasticity of plant processes, other pathways can be switched on if there is a block in the usual sequence of events. Therefore, the key controlling genes of senescence remain to be identified.

Value of Senescence Research

Senescence is the final stage of plant development and has a key role to play in generating sufficient reserves for the plant to survive, either in following seasons or in the next generations. Cereal plants that have been bred to remain

green longer have higher yields and increased tolerance to stress. Premature senescence induced by stress results in reduced yield and quality in crops. Post-harvest senescence also has a severe implication on nutrient quality of both forage crops and green vegetables. Thus, if we could identify and manipulate the key regulatory genes that control senescence many important agronomic improvements could be made that would be beneficial to growers and consumers.

Further Reading

- Buchanan-Wollaston V, Earl S, Harrison E *et al.* (2003) The molecular analysis of plant senescence – a genomics approach. *Plant Biotechnology Journal* **1**: 3–22.
- Buchanan-Wollaston V, Page T, Harrison E *et al.* (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation induced senescence in *Arabidopsis*. *Plant Journal* **42**: 7–585.
- Feller U and Fischer A (1994) Nitrogen metabolism in senescing leaves. *Critical Reviews of Plant Science* **13**: 41–273.
- Gan S and Amasino RM (1996) Cytokinins in plant senescence: from spray and pray to clone and play. *Bioessays* **18**: 557–565.
- Gan S (2007) Senescence processes in plants. *Annual Plant Reviews*, vol. 26. Ames, IA: Blackwell Publishing.
- Hortensteiner S (2006) Chlorophyll degradation during senescence. *Annual Review of Plant Biology* **57**: 55–77.
- Hörtensteiner S and Feller U (2002) Nitrogen metabolism and remobilization during senescence. *Journal of Experimental Botany* **53**: 927–937.
- Lim PO, Woo HR and Nam HG (2003) Molecular genetics of leaf senescence in *Arabidopsis*. *Trends in Plant Science* **8**: 272–278.
- Thomas H and Stoddart J (1980) Leaf senescence. *Annual Review of Plant Physiology* **31**: 83–111.
- Thomas H, Ougham H, Wagstaff C and Stead A (2003) Defining senescence and cell death. *Journal of Experimental Botany* **54**: 1127–1132.