Review

The molecular analysis of leaf senescence – a genomics approach

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Summary

Senescence in green plants is a complex and highly regulated process that occurs as part of plant development or can be prematurely induced by stress. In the last decade, the main focus of research has been on the identification of senescence mutants, as well as on genes that show enhanced expression during senescence. Analysis of these is beginning to expand our understanding of the processes by which senescence functions. Recent rapid advances in genomics resources, especially for the model plant species Arabidopsis, are providing scientists with a dazzling array of tools for the identification and functional analysis of the genes and pathways involved in senescence. In this review, we present the current understanding of the mechanisms by which plants control senescence and the processes that are involved.

Introduction

Senescence in plants is an essential developmental phase that is illustrated clearly by the dramatic colour changes that occur in the autumn in temperate regions of the world. Green leaves on trees and other perennial plants turn to yellow, orange and red before they eventually brown, die and are discarded from the plant. Annual plants such as grain crops undergo a similar process when they turn from green to golden as the grain ripens before harvest. This visible transformation is accompanied by active metabolic changes that result in much of the nutrients stored in the leaf during development being transferred to other parts of the plant. In the case of deciduous trees, nutrients are stored in specialized cells in the trunk, while for annual crops such as wheat, the nutrients form an essential part of the developing seeds. The process of senescence is therefore of key importance to ensure vigorous survival of the species in the following season.

The main purpose of senescence in plants is for mobilization and recycling. During the growth and development of green leaves, an organ packed with nutrients is produced. When the leaf is no longer required by the plant, the senescence process is induced and recycling of all the mobilizable nutrients occurs. The final stage of this process is leaf death, but this is actively delayed until all nutrients have been removed through the processes of developmental senescence. This is clearly illustrated by experiments that show that senescence is reversible. A leaf that is completely yellow that has mobilized the great majority of its nutrient content can be induced to re-green by various treatments (Thomas and Donnison, 2000; Zavaleta-Mancera et al., 1999). The chloroplasts recover structural features, synthesis of chloroplast proteins occurs and photosynthesis starts again. This shows that a senescing leaf is in control of its fate from the start to the finish.

As well as being a developmental process, senescence can be induced prematurely by environmental conditions. Plants cannot move far from an unfavourable environment and senescence is one of the mechanisms that they have evolved to cope with this. Certain parts of the plant can be sacrificed to enhance the chances of survival of the rest of the plant. For example, pathogen infection of a single leaf will result in the induction of senescence in that leaf. This allows the plant to move nutrients away from the developing pathogen, so as to reclaim the nutrients before the leaf is discarded to remove...
the source of infection from the vicinity of the still healthy parts of the plant. Other environmental stresses such as drought or nutrient limitation or oxidative stress caused by UVB irradiation and ozone can result in premature senescence, shortening the lifetime of individual leaves or indeed the whole plant. Senescence-like symptoms are also caused when green parts of the plant are removed and stored. Post-harvest yellowing and loss of nutrients in green vegetables are important economic problems and potential links between this process and developmental senescence will be discussed later in this review.

Many reviews have been written describing the processes that occur during leaf senescence (Buchanan-Wollaston, 1997; Chandlee, 2001; Gan and Amasino, 1997; Nam, 1997; Nooden, 1988; Quirino et al., 2000; Smart, 1994; Thomas and Stoddart, 1980). In this review we do not intend to cover the complete senescence phenomenon and will focus primarily on leaf senescence. We will briefly describe the senescence processes and then discuss some recent results that have been obtained, mostly using the model plant Arabidopsis, that are starting to throw some light on the mechanisms that control senescence. The genomics resources that are now available for Arabidopsis have allowed the rapid identification of novel genes and mutants and, in the next few years, this will considerably speed our acquisition of knowledge. At this time however, many of the mechanisms that occur during the senescence process remain a mystery and substantial research will be required before we can understand how this highly complex process is controlled.

**Arabidopsis as a model plant for senescence studies**

The extensive genomic resources available for Arabidopsis make it very attractive for the identification and functional analysis of senescence-regulated genes. The growth stages of Arabidopsis have been carefully defined, which allows an accurate sampling of materials for comparative analysis (Boyes et al., 2001). However, Arabidopsis may not be the ideal plant in which to study senescence since the leaves have a very short lifetime and senescence seems to start as soon as full expansion is reached (Stessman et al., 2002). Figure 1 illustrates this; levels of the large subunit of RUBISCO are already diminished as the leaf reaches its full size (Harrison et al., manuscript in preparation). Moreover, the correlation between the senescence of the individual rosette leaves may not be closely linked to the developmental stage of the plant (Noodén and Penney, 2001). In many plants, such as the pea, removal of the developing flowers and pods will significantly extend the life of the leaves (e.g. Pic et al., 2002) but in Arabidopsis, male sterile mutants, or plants from which the developing bolts were removed, did not show any extension to the lifetime of the individual leaves (Hensel et al., 1993). Therefore, Arabidopsis may not provide a good model for the study of developmental senescence. However, using a different Arabidopsis accession (Columbia instead of Landsberg) and looking specifically at one particular leaf, Ye et al. (2000) did show that leaf chlorosis was delayed in plants from which the developing bolts were constantly removed. The
developmental signals that initiate leaf senescence may be weaker in Arabidopsis, and ageing or stress may have a more significant role than in other plants. However, all normal senescence processes do occur in Arabidopsis: macromolecules are degraded and mobilization of N, P, etc. occurs with reasonable efficiency (Himelblau and Amasino, 2001). Therefore, much useful information on genes and gene regulation will be gained from the analysis of senescence in Arabidopsis, although testing genes in other species will be important to confirm the conclusions that are reached.

What happens in leaf senescence

The senescence process takes place in a highly regulated manner and the cell constituents are dismantled in an ordered progression. Chlorophyll degradation is the first visible symptom of senescence but by the time yellowing of the leaf can be seen, the majority of the senescence process has occurred. Protein and RNA degradation parallels a loss in photosynthetic activity; nutrients such as nitrogen, phosphorus, metal ions and minerals are transferred out of the leaf.

Chlorophyll degradation

A recent review by Hörtensteiner and Feller (2002) described the current understanding of chlorophyll and protein degradation during senescence. The pathway for chlorophyll degradation has been elucidated in the last few years (Matile et al., 1999) and a number of the genes in the pathway have been cloned. None of these however, show an enhanced expression during senescence (reviewed in Takamiya et al., 2000). The key enzyme in the pathway appears to be pheophorbide a oxygenase which cleaves the tetrapyrrole ring to produce RCC (red chlorophyll catabolite) (Hörtensteiner et al., 1998). A stay-green mutant of Festuca pratensis (sid) accumulates pheophorbide a and is assumed to have a defect in the gene encoding pheophorbide a oxygenase (Vicentini et al., 1995). The activity of pheophorbide a oxygenase increases dramatically during senescence, implicating this enzyme as a control point in the process. Cloning and characterization of this gene would be a key step in the elucidation of the control mechanisms for chlorophyll degradation. The final products of chlorophyll catabolism, known as non-fluorescent chlorophyll catabolites (NCCs) are deposited in the vacuole with no recycling of any of the nitrogen contained within them (Hinder et al., 1996; Tommasini et al., 1998). Therefore, the energy expensive chlorophyll degradation steps are not carried out in order to mobilize the nutrients, but take place to detoxify this highly reactive compound as it is released from the pigment–protein complexes. This is essential to maintain the viability of the plant cell while senescence is taking place. The importance of chlorophyll degradation has been well illustrated by the recent identification of the accelerated cell death 2 (Acd2) gene product as an enzyme involved in chlorophyll degradation (RCC reductase) (Mach et al., 2001). In the absence of this enzyme activity, the accumulation of phytotoxic chlorophyll products such as RCC causes rapid cell death.

Protein degradation

A major question in plant senescence is the conundrum of how the leaf protein, up to 75% of which is located within the chloroplast, is degraded and mobilized. In addition, what signals act to initiate this process? Many protease genes show induced expression during senescence (Table 1), but these appear to encode enzymes localized in the vacuole and are therefore not in contact with chloroplast proteins until the membranes disrupt late in senescence. There have been a number of reports to indicate that degradation of stromal proteins such as Rubisco and glutamine synthetase can be initiated non-enzymatically by reactive oxygen species (ROS) when chloroplasts are incubated in photo-oxidative stress conditions (Ishida et al., 1999, 2002; Roulin and Feller, 1998). However, it is not clear whether increased ROS could initiate the early degradation of Rubisco during senescence. Although ROS levels do increase during senescence, this is likely to be the result of macromolecule degradation processes and thus occur after protein and lipid degradation is initiated. There are reports of the activity of aminopeptidases and metalloendopeptidases in the chloroplast and also chloroplast localization of members of the Clp protease family (Roulin and Feller, 1998; Shanklin et al., 1995). These enzymes may have a role in protein turnover during leaf development but there is no clear evidence to show that they control protein degradation during senescence (Majeran et al., 2000; Shikanai et al., 2001). Degradation of thylakoid proteins such as LHCP II appears to follow a different route. This protein exists as a pigment–protein complex with chlorophyll and its degradation requires the parallel detoxification of the released chlorophyll, as described above. This is shown in the stay-green Festuca mutant where the catabolism of Chl is blocked, the LHCP protein is stabilized and does not get degraded (Thomas and Donnison, 2000). The first step in the degradation of proteins such as LHCP may be the removal of chlorophyll catabolites which destabilizes the protein complex, allowing degradation by chloroplast proteases. Vacuolar proteases may not have a role in protein degradation efficiency (Himelblau and Amasino, 2001). Therefore, much useful information on genes and gene regulation will be gained from the analysis of senescence in Arabidopsis, although testing genes in other species will be important to confirm the conclusions that are reached.

Molecular analysis of plant senescence

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Many senescence enhanced genes show similarity to other genes in the databases which allows a potential function to be assigned to them. This table illustrates a range of possible functions for some of the senescence enhanced genes that have been reported. This is only a representation of published results and many reports are not included.

degradation until the final lytic stages after the membranes have disrupted. There is evidence that some senescence-enhanced proteases accumulate in the vacuole as an inactive aggregate, which slowly matures to produce a soluble active enzyme at later stages of senescence (Yamada et al., 2001). The ubiquitin pathway for targeted protein degradation is important to control protein turnover during normal development. Increased expression of a polyubiquitin gene SEN3 has been detected in the senescing leaves of Arabidopsis (Park et al., 1998) indicating that ubiquitin dependent proteolysis may be an important aspect of non-chloroplast protein degradation during senescence. The recent analysis of the Arabidopsis delayed senescence mutant Ore9 showed that the ORE9 protein is an F-box protein which interacts with a component of the plant SCF complex which controls selective ubiquitination and subsequent proteolysis of target proteins (Woo et al., 2001). This result indicates that the ubiquitin-mediated degradation of specific proteins has an important role in the control of senescence. The authors postulate that the ORE9 protein may be involved in the degradation of a key regulatory repressor of senescence and the continuous presence of this may inhibit the onset of senescence. The identification of the proteins that are the targets for the ORE9 complex is therefore of great interest to improving our understanding of the control of senescence.

Lipid degradation
During senescence there is a decline in the structural and functional integrity of cellular membranes which is the result of the accelerated metabolism of membrane lipids (reviewed in Thompson et al., 1998). Enzymes such as phospholipase D, phosphatidic acid phosphatase, lytic acyl hydrolase and lipoxygenase have been implicated; certain genes that may encode enzymes with these activities show senescence-enhanced expression (Table 1; He and Gan, 2002; Thompson et al., 1998). Thylakoid membranes provide an abundant source of carbon that can be mobilized for use as an energy source during senescence. Enzymes that carry out peroxisomal fatty acid β-oxidation are present in mature leaves (Graham and Eastmond, 2002), and this pathway becomes important when carbohydrate supplies are depleted such as occurs during senescence. Senescence-enhanced expression of genes encoding enzymes required for β-oxidation and the glyoxylate pathway (Table 1; reviewed in Graham and Eastmond, 2002) indicates the importance of these pathways in lipid degradation and remobilization but the signals that induce these pathways during senescence have not been elucidated. The acetyl CoA derived from fatty acid breakdown can be used directly for respiration or, via the glyoxylate pathway and gluconeogenesis, can be used for the synthesis of sugars which may be exported. Senescence-enhanced expression of pyruvate orthophosphate dikinase (Table 1), which is required for the conversion of pyruvate to sugars via the gluconeogenesis pathway, indicates that this pathway does occur during senescence. The expression of a gene encoding a monosaccharide transporter may implicate this protein in the mobilization of sugars from the cell (Quirino et al., 2001).

There have been two recent reports indicating that senescence may be affected by altered levels of acyl hydrolase genes that could be involved in lipid degradation. Firstly, transgenic Arabidopsis plants that had reduced levels of a senescence enhanced lipase, originally identified in carnation petals (Hong et al., 2000), showed delayed leaf senescence (Thompson et al., 2000). A similar study with an acyl hydrolase gene from Arabidopsis (SAG101), showed that the antisense suppression of this gene delayed leaf senescence by a few days (He and Gan, 2002). In addition, over-expression of this gene accelerated the onset of senescence. It is not clear from these reports whether it is the same gene that is being studied; both enzymes had fairly weak acyl hydrolase activity when tested with an artificial substrate. The authors suggest that this enzyme might act to initiate the senescence-related degradation of membranes. Release of free fatty acids and the consequent perturbation of the lipid bilayer could make it susceptible to further degradation by other lipolytic enzymes. An alternative explanation for the altered senescence phenotype seen in the under- and over-expressing plants may be that α-linolenic acid released from the membranes by the action of this enzyme could be made available for the synthesis of jasmonic acid (JA). Altered levels of JA in the leaves could affect the initiation of senescence (see below).

Other events
Nucleic acids, especially RNA, form a valuable source of phosphorus in a mature leaf. Senescence-enhanced expression of genes encoding several different nucleases has been reported (Table 1), and these presumably act to degrade nucleic acids during senescence. Total RNA levels fall rapidly with the progress of senescence; nuclear DNA is maintained to allow gene expression to continue, until late in the process. Other valuable leaf constituents include metal ions such as K, Mo, Cu and Fe, and it is likely that much of these are also mobilized from leaves during senescence. In a recent paper,
Himmelblau and Amasino (2001) analysed nutrient mobilization from Arabidopsis leaves and showed that the levels of many compounds measured (Mo, Cr, S, Fe, Cu and Zn) were reduced by over 50% in senescent leaves when compared to green leaves. Levels of the valuable nutrients N, P and K were reduced by at least 80%. Little is known about the genes that encode enzymes that carry out the mobilization processes. Levels of cytosolic glutamine synthetase (GS) increase during senescence (Table 1); the role of this enzyme is likely to be in the conversion of amino acids to glutamine to increase the efficiency of nitrogen transport. Increased levels of glutamine have been measured in B. napus leaves and phloem in the late stages of senescence (Finnemann and Schjoerring, 2000). The cytosolic form of GS is predominantly located in the vascular bundles in senescing rice leaves and Schjoerring, 2000). 14-3-3 protein which increases enzyme activity (Finnemann et al, 1996) indicating its role in nitrogen transport. Transcript levels of GS1 increase during senescence, but post-translational control of GS activity has also been shown to occur by both phosphorylation, which protects the protein from degradation, and by interaction with 14-3-3 protein which increases enzyme activity (Finnemann and Schjoerring, 2000).

**Programmed cell death and senescence**

Plant PCD occurs in a wide range of different specialized situations including the development of tracheary elements (Fukuda, 1996), in the hypersensitive response to incompatible pathogens (HR) (Morel and Dangl, 1997) and in the formation of aerenchyma in waterlogged roots (Drew et al., 2000). In these cases, the cell is actively and rapidly killed for a defined structural or defence purpose. Senescence results in massive levels of cell death, but the purpose of senescence is not the death of the cell; rather death only occurs when senescence has been completed. Senescence occurs in two stages. The first stage is reversible and the cells remain viable throughout. The second stage results in cell death, which could be deliberately induced or may be necrosis resulting from an extensive weakening of cellular structures. Leaves on Arabidopsis and many other monocarpic plants are brown and dried in appearance when this stage has been reached. In other species – some deciduous trees for example – leaves may be lost from the plant before this necrotic stage is reached. This raises the question: Is PCD a part of the senescence process?

Controlled vacuolar collapse and chromatin degradation revealed by DNA laddering seem to be reliable markers to distinguish PCD from necrosis (Jones, 2001). DNA laddering has rarely been reliably detected in senescing leaves (Lee and Chen, 2002) and the majority of the senescence process has occurred well before vacuolar collapse takes place. However, there is some evidence to indicate that this final death phase of senescence is a regulated PCD process. Leaves from Arabidopsis pad4 mutants which are defective in the salicylic acid (SA) signalling pathway do not appear to undergo cell death as efficiently as the wild-type (Morris et al., 2000). Leaves often remain yellow without going necrotic. This result implicates the SA pathway in senescence-related cell death, which may occur via a similar mechanism to pathogen or ozone induced death, which can also be dependent on the SA pathway. (Rao and Davis, 2001). Expression of the SAG12 cysteine protease gene occurs in yellowing tissue late in senescence; by the time chlorosis is visible the majority of the protein has been degraded and mobilized from the cell. The requirement of this gene for the SA pathway for expression (see below) may implicate a role in cell death, or at least in the degradation of the final vestiges of cellular constituents in the last stages of senescence. Similarly, a metalloproteinase has been identified in senescing cucumber cotyledons which is also expressed very late in senescence (Delorme et al., 2000). The authors suggest that this protein, expressed too late for nutrient mobilization, may have a role in PCD.

**Genes involved in leaf senescence**

Biochemical and molecular studies over the last few decades have shown that senescence is an active process that requires the expression of novel genes and the synthesis of new proteins (Nooden et al., 1997). Genetic studies on leaf senescence have, in general, taken two different approaches. Genes with a role in leaf senescence can be identified by the isolation and characterization of mutants that are defective in some aspect of the senescence pathway. Alternatively, many research groups have used differential expression as a way of identifying senescence-enhanced genes. Functional analysis of these genes (often called SAGs (senescence associated genes), or more accurately, SEN genes (senescence enhanced)) is then necessary to indicate their role and importance in senescence.

**Study of mutants**

The first indication that the expression of novel genes was required for senescence came from the identification of senescence mutants. Early senescence modifying mutants include the sid (senescence induced degradation) mutant of Festuca pratensis which was described by Thomas (1987). Inheritance studies showed that this recessive stay-green
phenotype was due to a single nuclear locus, and recent studies have shown this to be either the gene encoding pheophorbide a oxygenase, or a regulator of this gene (see above). Moreover, the phenotype of the famous stay-green pea used by Mendel has been shown to be due to a mutation in this gene (Thomas et al., 1996). In these mutants, the chlorophyll degradation pathway is affected, but much of the senescence proceeds normally. A number of different soybean genotypes have been identified that show alterations in senescence. Homozygous d1d1d2d2 lines show similarity to the sid mutant, in that chlorophyll and chlorophyll binding proteins are maintained but photosynthesis declines (Guiamet et al., 1991). In the presence of an additional nuclear gene G (GGd1d1d2d2) the decline in photosynthetic capacity is prevented (Guiamet and Giannibelli, 1996). Senescence mutants in Arabidopsis have been elusive in spite of the vast resources of mutant lines that are available. The senescence mutants that have been identified, such as ore9 described above and mutants in the ethylene pathway (see below), tend to show a delay in the onset of senescence but no alteration in the senescence process itself once it has been initiated (Oh et al., 1997; Woo et al., 2001). However, Arabidopsis is probably not an ideal plant for the identification of mutants that do not undergo senescence; lack of senescence in the short-lived leaves could well be masked by the rapid onset of necrosis.

It has not been possible as yet to identify a mutant in a single gene that blocks all aspects of senescence. There are multiple signalling pathways involved in gene expression during senescence (see below), and it is quite likely that there is not a single regulatory gene that controls the whole process.

A mutant that showed an early senescence phenotype, hys 1 (hypersenescent 1) was recently identified (Yoshida et al., 2002). This mutant showed early loss of chlorophyll and expression of senescence enhanced genes and was found to be allelic with cpr5 (constitutive expresser of pathogenesis related genes 5). The HYS1 gene is expressed at all stages of leaf development and may encode a protein that represses the initiation of senescence. Alternatively, the enhanced levels of ROS, SA and other defence related components that result in the cpr5 phenotype may accelerate the onset of senescence (see below).

**Identification of senescence-enhanced genes**

Changes in the pattern of in vitro translation products from mRNA isolated from green or senescing leaves of a number of different plants showed that new RNA was synthesized during senescence (Buchanan-Wollaston, 1994; Davies and Grierson, 1989; Thomas et al., 1992). Over the last decade, research groups have applied a number of techniques aimed at the identification of genes that show enhanced expression during senescence. These techniques have included differential screening (Buchanan-Wollaston, 1994; Drake et al., 1996; Lohman et al., 1994; Park et al., 1998; Smart et al., 1995), subtractive hybridization (Buchanan-Wollaston and Ainsworth, 1997), differential display (Fujiki et al., 2001; Hajouj et al., 2000; Kleber-Janke and Krupinska, 1997; Yoshida et al., 2001) and suppressive subtractive hybridization (Hinderhofer and Zentgraf, 2001). We have recently used the technique of cDNA-AFLP (Bachem et al., 1996) to identify genes expressed in post-harvest broccoli heads (Page et al., 2001) and in leaf senescence in Arabidopsis (Figure 2). The advantage of the cDNA-AFLP technique is that several different RNA samples can be screened at once to identify genes more specific to senescence. For example, we used this technique to screen RNA isolated from the senescing leaves of several mutants to identify genes whose expression during senescence was not dependent on the SA signalling pathway (Figure 2B). A list illustrating the range of senescence-enhanced genes that have been identified to date is shown in Table 1. In addition to these, many genes that have been characterized encode proteins whose function is, as yet, unknown.

The availability of cDNA microarrays and Affymetrix GeneChips has considerably increased the speed by which differentially expressed genes can be identified in Arabidopsis. Chen et al. (2002) used Affymetrix GeneChips to analyse the expression of genes encoding different classes of transcription factor during a range of different stress treatments including different types of pathogen, cold, high salt and also during leaf development. They report the senescence-enhanced expression of over 40 different transcription factors. Most of these also showed induced expression in response to at least one stress treatment, which reflects the extensive overlap between gene expression during senescence and in stress responses (see below).

We have recently carried out an experiment using the same Affymetrix Arabidopsis GeneChip which carries probes for around 8000 Arabidopsis genes (Zhu and Wang, 2000). In a simple experiment comparing gene expression levels at three stages of leaf development (MG (mature green); S1 (early senescence, no chlorosis) and S2 (mid senescence, 5–15% chlorosis), we have identified over 1400 genes that show relative changes in expression during leaf development. Figure 3 shows the clustering pattern for these genes. The genes can be classed into at least four different groups depending on their expression patterns.
Group 1 represents genes that show enhanced expression early in senescence which is maintained at a high level at the S2 stage. Novel genes in this group encode a number of potential regulatory factors such as a MYB protein, a zinc finger protein, a protein phosphatase and a protein kinase, all of which may have a role early in senescence. Other genes in this group include a glutathione-S-conjugate transporter and an ATP sulphurylase precursor. Group 2 genes are expressed at a similar level in MG and S1 stages but expression levels increase at S2. Genes such as SAG12 fall into this group as well as potential cell wall degrading enzymes such as exopolygalacturonase, a pectinesterase and a β-glucosidase. Potential regulators such as a receptor kinase gene and a zinc finger are also in this group. Group 3 genes are expressed at higher levels in green leaves, with expression falling during senescence; genes encoding proteins such as RBCS and chlorophyll a/b binding protein are in this group. In addition, a putative expansin, a photosystem 1 reaction centre protein and a protochlorophyllide reductase precursor fall into this group.

An alternative approach to the identification of senescence enhanced genes has been to use the enhancer trap libraries that are available for Arabidopsis. He et al. (2001) screened 1300 enhancer trap lines and identified 147 that showed GUS expression in senescing leaves but not in green leaves from the same plant. The DNA flanking the insertion site of three of these genes was cloned and, when tested by Northern hybridization, showed senescence-enhanced expression. SAG101, the acyl hydrolase gene described above, was identified by this method (He and Gan, 2002). There are several advantages to using this approach for gene identification; having the reporter gene linked to the regulated promoter

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**Figure 2** Expression analysis of genes identified by cDNA-AFLP. Potential senescence-enhanced genes from Arabidopsis leaves were identified by cDNA-AFLP and their expression was then confirmed using virtual Northern hybridization analysis as described in Page et al. (2001). Genes are named LSC (Leaf Senescence Clone). (A) Genes identified using RNA isolated from green (MG), early senescent (S1) and mid-senescent (S2) leaves. Gene chromosomal location numbers are: LSC326, At4g32940; LSC332, At2g17710; LSC333, At3g11530; LSC334, At4g21580; LSC335, At1g27020; LSC336, At2g04110; LSC337, At2g18050 and LSC322, At2g39010. (B) Genes identified using a wider range of RNA samples in the cDNA-AFLP experiment. The aim of this experiment was to identify genes that were expressed at wild-type levels in senescing leaves of plants defective in the SA pathway. RNA was isolated from MG, S1 and S2, as above as wells as from senescing leaves (S2) from NahG transgenic plants and npr1 and pad4 mutants (see Morris et al., 2000). Gene chromosomal location numbers are: LSC328, At1g76680; LSC323, At5g03290; LSC340, At2g16860; LSC324, At1g20220 and LSC327, At2g27020. (note LSC327 and LSC335 represent the same gene, identified in two different experiments).

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**Figure 3** Gene expression profiles during Arabidopsis leaf development. Expression data from Affymetrix GeneChip experiments was normalized and relative expression levels determined. Genes showing similar expression levels in all samples were filtered out as well as genes with very low expression in all samples. The remaining data (for n = 1400 genes) was subjected to a GENETREE clustering (using GeneSpring software, Silicon Genetics, CA). Red shows relatively high expression and blue indicates low expression. The clustering analysis indicates at least four groupings by expression pattern and these are indicated by horizontal lines. The four small graphs show four clusters identified by K-means clustering which represent the different expression patterns. The colour of the lines in these graphs is related to expression levels in the S2 sample.
allows a rapid assessment of the other sites in the plant in which the gene is expressed. He and co-workers showed that many (but not all) of the genes they had identified were also expressed in senescing flowers, stems and siliques, while some were expressed constitutively in the other organs. In addition, they used the expression lines to examine regulatory pathways. Potential senescence-enhancing treatments such as abscisic acid (ABA), JA, ethylene and darkness were used to show that the genes could be divided into groups depending on the signals that controlled their expression, and this allowed a putative network of signalling pathways to be proposed. Interestingly, the majority of the genes examined did not respond to any of these treatments, indicating that they might be more specific to senescence.

Therefore, a large number of senescence-enhanced genes have now been identified, particularly from Arabidopsis. The availability of complete genome arrays for Arabidopsis will rapidly extend this number in the near future. Hopefully, similar arrays will soon be available for other crops and this will allow us to compare and contrast genes expressed during senescence in different plant species.

Functional analysis of senescence-enhanced genes

The identification of genes that show altered expression during senescence is useful for several purposes. Firstly, genes that show enhanced expression can be assumed to encode proteins that have a role to play in the senescence process. The availability of the complete Arabidopsis sequence allows the structure of each gene to be predicted and in many cases the likely activity of the protein can be assumed. However, in only a few cases has that role been proved by functional analysis.

The use of the rapidly extending libraries of Arabidopsis insertion mutants (e.g. the T-DNA insertion lines from the Salk Institute Genomic Analysis Laboratory <http://signal.salk.edu/cgi-bin/tdnaexpress>) will allow a functional analysis of individual genes to be carried out more rapidly. Currently, T-DNA insertions have been identified in many Arabidopsis genes, and seed containing many of these can be obtained from stock centres. In essential genes, insertions will be lethal and will therefore not be available. There may be genes that have a role in senescence but are also essential in early stages of development. For example, genes that are required for the mobilization of fatty acids in a germinating seed could also have a role in senescence. A functional analysis of these will require a more specific approach. The use of antisense or RNAi to reduce the expression of individual genes using a senescence-specific promoter to express the inhibitory RNA may be required to find the senescence role of some genes. Again, Arabidopsis has an advantage for this analysis. The rapid generation of RNAi lines is now possible using Gateway technologies to construct transformation vectors (Karima et al., 2002; Wesley et al., 2001). This can be followed by Arabidopsis transformation using floral dip methods which are rapid and relatively non-labour intensive (Clough and Bent, 1998).

Another potential problem with the insertion mutant approach for functional analysis is the possible functional homologues of particular genes. The sequencing of the Arabidopsis genome revealed large areas of duplication that may contain genes that carry out the same function (Blanc et al., 2000). Insertion knock-out of one of these duplicates would not show any phenotype. For example, the shatterproof genes SHP1 and SHP2, which are in a chromosomal block that duplicated around 100 million years ago (Vision et al., 2000), must be removed simultaneously before the non-dehiscence phenotype is observed (Liljegren et al., 2000). Using RNAi with the cloned fragment selected to be similar to both functional homologues could be a useful method for removing the activity of both genes at once.

Having an extensive collection of genes that show altered expression patterns during senescence provides a very useful tool for the functional analysis of potential regulatory genes. Knock-out mutants can be obtained for potential transcription factor or other regulatory genes and gene expression patterns in the mutant compared to the wild-type. We have generated microarrays carrying around 600 different senescence or stress related genes. Using these arrays (and more extensive arrays that will be constructed as more senescence enhanced genes are identified), we can examine the effects of mutations that may alter transcription and signalling during senescence. Figure 4 illustrates the use of cDNA microarrays for this analysis. These arrays have been probed with RNA extracted from the senescing leaves of wild-type plants and plants that are defective in the salicylic acid (SA) signalling pathway. Relative expression levels (wild-type : mutant ratios) can be used to show the effects of the defect on gene expression. In this example, the transgenic NahG line has been used, but this approach is also being used in our group to analyse knock-out mutants or transgenic RNAi lines for selected senescence-enhanced regulatory factors. The NahG gene encodes an enzyme that degrades SA and hence these plants do not express any genes that require SA for expression. From this array we can see that the SA pathway is involved in the expression of a number of genes during senescence (Morris et al., 2000; see below).
whether this homeobox gene, which is normally expressed from the same senescence specific promoter showed a similar phenotype and increased levels of cytokinin in the leaves of these transgenic plants, including chlorophyll degradation, protein degradation and loss of photosynthetic status (Wingler et al., 1998). This indicates that the senescence process is delayed at an early stage. Transgenic tobacco plants in which the maize homeobox gene knotted1 was expressed from the same senescence specific promoter showed a similar phenotype and increased levels of cytokinin in the older leaves (Ori et al., 1999). However, it is not clear whether this homeobox gene, which is normally expressed in developing meristems and not in leaves, has a role in controlling senescence in normal plants. In an activation tagging experiment in petunia, delayed senescence has been shown in a tagged line that over-expresses Sho, a gene that has similarity to IPT genes from Arabidopsis and is therefore likely to encode a cytokinin biosynthesis gene (Zubko et al., 2002). Premature senescence was detected in Arabidopsis lines which over-expressed the mevalonic pathway gene, farnesyl diphosphate synthase (FPS) (Masferrer et al., 2002). Overproduction of FPS led to a reduction in the substrates required for cytokinin biosynthesis and a consequent reduction in cytokinin levels presumably led to the premature senescence-like symptoms and early expression of SAG12 that was reported.

Ethylene is essential for the ripening of many fruit, but it has also been shown to have a role in leaf senescence in some plants. Plants that are exposed to ethylene show premature senescence and the older leaves on the plant are induced to yellowing. The leaves of an ethylene insensitive mutant of Arabidopsis (Etr1) were delayed in their onset of senescence (Grbic and Bleecker, 1995); similarly, an antisense tomato plant that synthesized very low levels of ethylene showed delayed leaf senescence (Picton et al., 1993). In addition, certain Arabidopsis mutant lines that have been identified as showing delayed senescence turn out to have defects in genes in the ethylene signalling pathway (Oh et al., 1997). However, in all these cases senescence occurs normally once the process has begun. Hence, it has been concluded that ethylene is a modulator of leaf senescence; its presence will speed up the senescence process but it is not essential for senescence to occur. Leaves have to be a certain age to be ready for the ethylene signal, young leaves treated with ethylene do not senesce.

Figure 4 Using cDNA microarrays to identify differentially expressed genes in Arabidopsis leaves. cDNAs representing senescence enhanced and control genes were spotted on to glass slides which were simultaneously probed with first strand cDNA made from RNA isolated from wild-type plants (Cy3) or NahG transgenic plants (Cy5). Gene expression levels in the wild-type are shown by scanning the slide for Cy3 fluorescence and in the NahG plant by scanning for Cy5 fluorescence. A small portion of the array is shown – the genes are loaded in duplicate pairs. Intensity of colour reflects levels of expression with white the highest, through red, orange, yellow, green, turquoise, bright blue to dark blue. Most genes are expressed at the same level in both treatments but a few, shown by boxes, are reduced in expression in the NahG plant. In this way, the effects of a mutation or transgenic alteration on the expression of a large number of genes can be simultaneously determined.

**Regulation of leaf senescence**

**Hormonal control of senescence**

Senescence in leaves is induced by many different factors and it is obvious that there are many different pathways involved in controlling the process (Gan and Amasino, 1997; Nam, 1997). The best evidence for hormonal involvement in the control of senescence is for the hormones cytokinin and ethylene. The signal that initiates the onset of developmental senescence appears to involve cytokinin. It has been known for many years that treatment with cytokinin can delay leaf senescence, and several reports in the last few years have illustrated the importance of cytokinin in the control of senescence. Tobacco plants that express a cytokinin biosynthesis gene (the Agrobacterium ipt gene) from a senescence-enhanced promoter (SAG12) were shown to remain green and non-senescent for an extended period of time (Gan and Amasino, 1995). All aspects of senescence are delayed in the leaves of these transgenic plants, including chlorophyll degradation, protein degradation and loss of photosynthetic status (Wingler et al., 1998). This indicates that the senescence process is delayed at an early stage. Transgenic tobacco plants in which the maize homeobox gene knotted1 was expressed from the same senescence specific promoter showed a similar phenotype and increased levels of cytokinin in the older leaves (Ori et al., 1999). However, it is not clear whether this homeobox gene, which is normally expressed...
senescence. However, in most cases, stress response pathways appear to be involved after the onset of senescence, indicating that they occur downstream of the senescence induction signal. Links between gene expression and pathogen responses were initially indicated by the discovery that pathogenesis-related (PR) genes (genes that are expressed in response to pathogens) are expressed during the senescence of healthy leaves (Hanfrey et al., 1996; Quirino et al., 1999). Conversely, genes identified as being senescence-enhanced have been shown to be expressed in leaves exposed to many different stresses such as pathogen infection (Butt et al., 1998; Pontier et al., 1999), ozone treatment (Miller et al., 1999), UV-B exposure (John et al., 2001, and see below) and others. A number of different senescence enhanced genes were induced in response to different stresses, but they did not all show the same patterns of expression (Park et al., 1998; Weaver et al., 1998). The GeneChip experiments of Chen et al. (2002), described above have shown that many common regulatory factors are expressed in senescence and in stress responses. Overall, it can be concluded that many signalling pathways control gene expression in response to different stresses and some of these are involved in leaf senescence in Arabidopsis.

The signalling molecules SA, JA and ethylene have been implicated in complex interconnecting pathways that control gene expression in plant pathogen responses as well as in plant responses to stress (reviewed in Turner et al., 2002; Wang et al., 2002). These pathways may also be involved in regulating gene expression during senescence. Morris et al. (2000) showed that the expression of certain genes during leaf senescence depended on the presence of an active SA pathway, and this is also illustrated in Figure 4. SA levels were shown to increase in senescing leaves and this increase could account for the senescence-enhanced expression of some of the genes examined. Genes encoding the PR proteins PR1a and a chitinase are also induced in SA treated green leaves. In contrast, expression of SAG12, a senescence-specific cysteine protease (Lohman et al., 1994), requires the SA pathway for senescence-enhanced expression but is not expressed in SA treated green leaves. Expression of this gene during senescence therefore requires the presence of an ‘age’ related factor in addition to SA (Morris et al., 2000). Senescence appears to occur normally in SA deficient plants, indicating that the genes controlled by SA are not essential for senescence. However, there may be some role for this pathway in the final death phase of senescence (see above).

Jasmonic acid (JA) and related compounds play an important role in regulating a number of plant responses such as wounding and pathogen infection (reviewed in Turner et al., 2002). JA has also been implicated in senescence. It was shown that exogenous treatment of barley leaves with JA or MeJA (methyl jasmonate) led to a loss of chlorophyll and reduced levels of RBCS, indicating that senescence was induced (Parthier, 1990). Recently, the role of the JA pathway in senescence has been investigated in Arabidopsis (He et al., 2002). These authors showed that treatment of Arabidopsis with JA resulted in typical premature senescence symptoms, and these did not occur on the JA insensitive mutant Coi1 (Xie et al., 1998). In addition, JA levels were shown to increase during senescence and several enzymes involved in JA biosynthesis showed senescence-enhanced expression. Studies on genes that may be involved in the chlorophyll degradation pathway have shown that the expression of a potential chlorophyllase gene (AtCHL1), is enhanced by MeJA treatment (Tsuchiya et al., 1999). However, senescence in the JA insensitive mutant Coi1, or in plants which produce low levels of JA due to a knock-out mutation in OPR3, does not appear to be impaired (He et al., 2002; Stintzi and Browse, 2000). Therefore, as is the case for the SA pathway and ethylene resistant mutants described above, although the JA pathway appears to have a role in senescence, it is not essential for the process to take place. Neither is it essential for the timing of the process.

Increased levels of ROS (reactive oxygen species) are a common factor between different stress responses as well as in senescence. Macromolecule degradation is likely to increase the levels of ROS in senescing tissues (del Rio et al., 1998; Thompson et al., 1998) and lipid peroxidation products increase in senescing Arabidopsis leaves (John et al., 2001; Ye et al., 2000). We have shown that the expression of many different senescence enhanced genes is induced in green leaves treated with silver nitrate; co-treatment with the ROS quencher, ascorbate, attenuated that expression, indicating that ROS were involved (Navabpour et al., manuscript in preparation). A similar result was obtained following treatment with 3-amino triazole, a catalase inhibitor. Therefore, the enhanced expression of certain genes during senescence may be mediated by increased ROS levels and this could also account for the expression of these genes in response to stress treatments such as ozone or pathogen infection. The tissues treated with silver nitrate undergo a cell death-like process. They do not enter senescence, since many senescence-enhanced genes are not expressed.

How similar are the events that occur in stress-induced senescence to those that take place in developmental senescence? To address this question, we have used an Affymetrix GeneChip experiment to compare gene expression in response
to UVB irradiation with that seen during developmental senescence. *Arabidopsis* plants treated with UV-B show senescence-like symptoms (Figure 5A), chlorophyll levels drop and photosynthesis rates decrease. UV-B irradiation results in increased ROS (Surplus *et al.*, 1998) and there is also an indication that senescence is induced by this treatment (John *et al.*, 2001). We have recently shown that many of the treated leaves recover from the stress, that gene expression reverts close to that seen before treatment and that photosynthesis is restored (Earl *et al.*, manuscript in preparation). This result also indicates that senescence is being induced rather than cell death. The Affymetrix data indicates that, although there are many genes that show a similar expression pattern in both senescence and in UV-B responses, there are also groups that show different expression patterns (Figure 5B).

Therefore, many signalling pathways are common between stress responses and senescence but there are some pathways that may be senescence specific. In addition, different stress treatments may well result in the induction of other signalling pathways not involved with the UV-B stress response. Dissection of the components of the signalling pathways that are senescence specific will identify genes that regulate the metabolic events that are unique to senescence.

**Sugar signalling**

Stress response pathways obviously have a role in the senescence process, but this may be of relatively low importance compared to the signalling pathways that initiate the onset of senescence and control the degradative processes that occur. Sugar sensing of signalling pathways have been implicated in the control of aspects of plant metabolism and development including plant senescence, and a central role for hexokinase as a glucose sensor modulating multiple signalling pathways has been proposed (reviewed in Rolland *et al.*, 2002). Transgenic plants over-expressing hexokinase exhibit premature senescence, indicating that this protein may have a role in controlling senescence (Dai *et al.*, 1999; Xiao *et al.*, 2000). Reduced photosynthetic activity is an early event in senescence and is accompanied by a loss of protein, followed by chlorophyll and lipid degradation. Senescence is also induced in dark treated tissues, where sugar levels rapidly become diminished. It has been postulated that the reduction in efficiency of photosynthesis, resulting in sugar starvation in the leaf, may be an early signal for the induction of senescence (Hensel *et al.*, 1993). This is supported by the observation that the dark induced expression of many senescence-enhanced genes is repressed in the presence of sucrose (Chung *et al.*, 1997; Fujiki *et al.*, 2001). Interestingly, sugar treatment of *Arabidopsis* leaves that were in a mid-senescent stage reduced the expression of the senescence specific gene, SAG12 but not another senescence enhanced gene SAG13 (Noh and Amasino, 1999). However, in green leaves, low sugar levels enhance photosynthesis, and the accumulation of glucose and sucrose represses the transcription of photosynthetic genes (Rolland *et al.*, 2002). Moreover, senescing leaves have been found to accumulate glucose and fructose, rather than exhibiting sugar starvation (Stessman *et al.*, 2002; Wingler *et al.*, 1998). The *hys1* mutant (described above) has an increased sensitivity to exogenously applied sugars as well as showing accelerated senescence, and the authors suggest that this gene might have a role in a sugar sensing pathway controlling the onset of senescence (Yoshida *et al.*, 2002). It is obvious that the relationship between sugar levels and senescence is not at all clear. Sugar may be involved in the regulation of some genes during senescence, but a combination of other developmental signals must also be involved (Ono *et al.*, 2001).

**Regulatory genes and promoters**

The identification of regulatory genes that could be manipulated to regulate the onset or progression of senescence is of
key interest and importance. Initially, the approach to this has been the isolation of senescence-enhanced genes, although early signal receptors or transcriptional repressors are unlikely to fall into this class. In the differential and other screen that have been applied to identify senescence enhanced genes (described above), a number of groups have identified potential regulatory genes. Genes that encode receptor kinase proteins that may serve as receivers or transducers of external or external signals, or genes that may encode transcription factors have been isolated (Table 1). A senescence-associated receptor kinase (SARK) gene, identified in bean, was shown to be expressed early in leaf senescence before the first signs of chlorophyll degradation or loss of chlorophyll a/b binding protein (Hajouj et al., 2000). This early expression implicates this protein as having a role in the early steps in senescence initiation rather than in downstream stress-related pathways.

Several classes of senescence-enhanced transcription factors have been isolated. A member of the Arabidopsis WRKY family of transcription factors, WRKY6, showed strong expression during leaf senescence (Robatzek and Somssich, 2001). Senescence and pathogen induced expression of the PR1 gene depended on the presence of the WRKY6 protein, which also appears to induce expression of NPR1, the transcriptional regulator required for PR1a expression (Zhou et al., 2000). A senescence specific receptor kinase gene, SIRK, that was dependent on WRKY6 for expression was also identified (Robatzek and Somssich, 2002). Although WRKY6 itself has a role in pathogen defence as well as senescence, the SIRK gene appeared to be expressed solely during senescence. The WRKY6 knock-out mutant resulted in an altered expression of several genes but no obvious senescence-related phenotype was observed. Another WRKY gene, WRKY53, was identified after a suppressive subtractive hybridization experiment designed to isolate genes expressed early in senescence (Hinderhofer and Zentgraf, 2001). This gene showed an interesting pattern of expression. It was expressed only in the oldest leaves of a 6-week-old plant but by the 7th week, expression was detected in all the rosette leaves, independent of their age. This implies that the gene is initially controlled by an age-related signal which is masked by a different signal when the plant reaches a certain developmental stage.

Two members of the leucine zipper (b-ZIP) family of transcription factors that show senescence-enhanced expression in tobacco have been identified (Yang et al., 2001). One of the genes, TBZF, was expressed in senescing leaves and flowers while the other, TBZ17, only accumulated in ageing leaves. The proteins were shown to accumulate in the guard cells and vascular tissues of senescing leaves. Both these cell types need to remain active until the very last stages of senescence, the guard cells to allow responses to environmental stimuli and the vascular tissues to maximize mobilization from the senescing leaf. The authors suggest that these genes may retard senescence by activating the genes that are required to retain cellular activity in these specific cell types.

The array experiments of Chen et al. (2002), described above identified many transcription factors that showed senescence-enhanced expression. In addition, we have identified around 40 different senescence-enhanced genes encoding proteins such as MYB, zinc finger, MADS box and leucine zipper as well as a number of different kinases. The functional analysis of these genes, to find the signalling pathway they are a component of and the downstream gene(s) that they regulate, will help to elucidate the complex pathways that control senescence. This is a key challenge for the next few years.

Little progress has been made in the identification of a ‘Senescence Box’, i.e. an upstream sequence that is required for senescence activated transcription. The involvement of multiple signalling pathways complicates the search for this sequence. Increased knowledge of individual pathways and the identification of smaller groups of genes that require the activity of a specific pathway for expression will increase the chances of identifying common senescence promoter sequences. A sequence identified in the upstream region of the Arabidopsis SAG12 gene was shown to bind different proteins in extracts from young or old leaves (Noh and Amasino, 1999). This result suggests that a repressor or inactive transcriptional activator may inhibit SAG12 expression in young leaves, to be replaced by a newly transcribed or modified activator during senescence.

A relatively simple model designed to illustrate the complexities of the signals and pathways that have a role in the control of gene expression during senescence is presented in Figure 6. There are obviously many other signalling factors and hormones that are not considered here, and multiple combinations of factors are possible. In addition, the relative importance of the particular pathways may well vary in different plant species.

Applications of senescence research in crop improvement

An increased understanding of the genes that control plant senescence is very important for future agronomic improvements in many crop species. Delaying senescence, particularly of the flag leaf, in grain crops such as wheat or maize would help to increase the grain yield and stay-green varieties are
used in some cereal improvement programmes (Borrell et al., 2001; Thomas and Howarth, 2000). Premature senescence induced by stress also has a detrimental effect on yield, and stay-green plants can exhibit enhanced stress resistance. In addition, the loss of quality caused by post-harvest induced senescence (yellowing) has a severe effect on the shelf life of green vegetables. Delaying yellowing would reduce wastage throughout the supply chain.

Gan and Amasino (1995) reported that the transgenic tobacco plants that showed autoregulated synthesis of cytokinin from the SAG12 promoter had an increased leaf number and seed yield. The possible benefits for this in agriculture could be substantial. Further analysis of these plants has confirmed that the older leaves remain green and photosynthetically active for longer, although they show a steady decline in protein and chlorophyll content (Wingler...
et al., 1998), indicating that additional factors have a role in controlling senescence. A further study by Jordi et al. (2000), using nitrogen-limited growth conditions, showed that chlorophyll levels were maintained in senescing leaves on the transgenic plants but soluble protein levels and photosynthetic activity were not very different from wild-type. Interestingly, the development of the younger leaves on the plant was severely affected by the delayed senescence exhibited by the older leaves. Inhibition of mobilization from older leaves resulted in reduced nitrogen and lower rates of photosynthesis in the young leaves. This would reduce the efficient use of light and nutrients. Therefore, it appears that, although the regulated synthesis of cytokinin can effectively delay senescence, the control of this synthesis must be finely balanced for agronomic benefits to be achieved.

The use of a similar system to improve stress tolerance has been reported recently. *Arabidopsis* transformed with the same SAG12:ipt fusion showed delayed senescence as well as an increased tolerance to flooding (Zhang et al., 2000).

**Post-harvest senescence in green vegetables**

Vegetables that are harvested when they are immature, before growth has ceased, are subjected to considerable stress due to the sudden disruption in energy, nutrient and hormone supplies (Huber, 1987; King et al., 1990). Consequently, produce such as asparagus and broccoli, senescence rapidly on storage and has a very short shelf life. Many of the changes seen during the storage of green vegetables, such as loss of chlorophyll, deterioration of cellular structure and, finally, cell death, show similarities to changes seen during developmental leaf senescence. We have found that many leaf senescence genes are also expressed in post-harvest broccoli, which indicates that many of the same pathways leading to senescence are likely to be involved (Page et al., 2001).

Knowledge of the hormonal control of senescence, gained from leaf senescence studies in *Arabidopsis* and tomato, has been used to manipulate post-harvest senescence in crops such as broccoli and lettuce. Using the SAG12 promoter fused to the Agrobacterium *ipt* gene as described above (Gan and Amasino, 1995), delayed yellowing has been shown in lettuce both before and after harvest (McCabe et al., 2001). Moreover, this construct has been transformed into broccoli and there is some evidence that post-harvest yellowing is also delayed in this species (Chen et al., 2001). Ethylene levels increase in broccoli florets post-harvest and the enhanced expression of genes encoding ethylene biosynthesis genes has been seen (Pogson et al., 1995). Transgenic broccoli containing an antisense ACC oxidase gene showed significant reduction in ethylene production and improvement in head colour changes after harvest (Henzi et al., 2000).

In our lab, we are investigating the genetic variation that controls shelf life in broccoli. A number of doubled haploid (DH) lines of broccoli were generated, many of them from the popular commercial cultivar Marathon. Doubled haploid lines were generated for these experiment because they are homozygous for all genes. Seed produced from these lines will have identical genetic makeup since there will be no segregation in the gametes produced. F1 plants derived from the crosses between DH parents will have a simpler genetic makeup, producing gametes in which one or other parental genotype segregates at each locus.

Shelf life assessments of these DH lines indicated a wide variation in their storage quality (Figure 7A). The best doubled haploid line performed better than Marathon, while the worst showed yellowing after only 2 days of storage (Figure 7B). This genetic difference will allow us to identify quantitative trait loci (QTL) that are associated with shelf life traits, and we are currently generating a DH mapping population in order to carry out a QTL analysis. The close genetic links between *Brassica* and *Arabidopsis* will allow the exploitation of *Arabidopsis* genomic information for the identification and analysis of the senescence-related genes within this region (Paterson et al., 2001). Thus, the exploitation of natural allelic variation is an alternative approach which can be used to identify the genes that have an important role in the control of senescence.

**Conclusions**

Our knowledge of the senescence system has expanded considerably in the last decade and, with the current advances in *Arabidopsis* resources, isolation of senescence-enhanced genes and elucidation of their roles in senescence will advance rapidly. This will result in exciting and new information in the next few years. However, beyond the transcriptome we remain quite ignorant of many of the events that occur during senescence. Signal receptors and repressors may not be identified as senescence enhanced genes, and alternative approaches, involving protein interactions with other proteins, nucleic acids or signalling molecules, or identification of novel mutants, will be needed to find these and analyse their functions. The post-transcriptional modification of proteins and the roles of metabolites and signalling factors will also need to be addressed. Technologies are rapidly improving for these types of analysis and applying these to the study of senescence is the challenge for the next decade.
An extension of these studies into crop plants, including the exploitation of genetic variation, will also be essential. Understanding how senescence is controlled in different crops will allow a future manipulation of senescence, either through genetic improvement or through the manipulation of key environmental triggers, with great potential benefits throughout the supply chain from producer to consumer.

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