Living to Die and Dying to Live: The Survival Strategy behind Leaf Senescence[1]

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Senescence represents the final developmental act of the leaf, during which the leaf cell is dismantled in a coordinated manner to remobilize nutrients and to secure reproductive success. The process of senescence provides the plant with phenotypic plasticity to help it adapt to adverse environmental conditions. Here, we provide a comprehensive overview of the factors and mechanisms that control the onset of senescence. We explain how the competence to senesce is established during leaf development, as depicted by the senescence window model. We also discuss the mechanisms by which phytohormones and environmental stresses control senescence as well as the impact of source-sink relationships on plant yield and stress tolerance. In addition, we discuss the role of senescence as a strategy for stress adaptation and how crop production and food quality could benefit from engineering or breeding crops with altered onset of senescence.

It does not take an expert's eye to notice how plant senescence is manifested in our daily lives. Senescence limits the shelf life of fresh vegetables, fruits, and flowers, implying that it is detrimental to survival. However, from the plant's perspective, senescence supports plant growth, differentiation, adaptation, survival, and reproduction (Thomas, 2013). Senescence is under strict genetic control, which is crucial for the plant's nutrient use efficiency and reproductive success. Senescence represents a major agricultural trait that affects crop yield and grain quality during food and feed production.

During senescence, mesophyll cells are dismantled in a programmed manner, undergoing changes in cell structure, metabolism, and gene expression. Ultrastructural studies have shown that chloroplasts are the first organelles to be dismantled (Dodge, 1970), while mitochondria and the nucleus remain intact until the final stages of leaf senescence (Butler, 1967). The salvaging of the chloroplasts allows a major portion of leaf lipids and proteins to be recycled (Ischebeck et al., 2006). As chloroplasts contain the majority of leaf proteins, they represent a rich source of nitrogen (N), and their salvaging provides up to 80% of the final N content of grains (Girondé et al., 2015).

During senescence, autotrophic carbon metabolism of the leaf is replaced by catabolism of cellular organelles and macromolecules. Metabolic profiling studies have revealed that N-containing and branched-chain amino acids accumulate in senescing leaves (Masclaux et al., 2000; Schippers et al., 2008). Interestingly, plants undergoing carbohydrate limitation metabolize proteins as alternative respiratory substrates (Araújo et al., 2011). Thus, to some extent, the availability of free amino acids ensures the maintenance of energy homeostasis in the senescing leaf, while these amino acids are also transported to sink tissues such as grains to support protein synthesis and N storage.

In addition to N remobilization, senescing leaves also undergo extensive lipid turnover. In both monocot and dicot plants, the total fatty acid content of senescing leaves decreases by at least 80% (Yang and Ohlrogge, 2009). Upon senescence, lipid synthesis rates are reduced, while the peroxisomal β-oxidation pathway is up-regulated (Christiansen and Gregersen, 2014). In Arabidopsis (*Arabidopsis thaliana*), remobilization of chloroplast lipids is essential for normal plant growth, the onset of senescence, and reproductive success (Padham et al., 2007).

Phosphate is a major component of plant fertilizers used in high-yield agriculture. In general, soil phosphate levels are suboptimal. Therefore, plants have evolved efficient mechanisms to remobilize stored phosphate during senescence (Himelblau and Amasino, 2001). Phosphate is remobilized through the degradation of organellar DNA and RNA as well as cytosolic ribosomal RNA. As decreased phosphate remobilization reduces total phosphate levels in seeds as well as seed

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germination rates (Robinson et al., 2012), senescence is crucial for seed viability. Furthermore, micronutrients such as zinc, iron, and molybdenum are strongly redistributed during senescence (Himelblau and Amasino, 2001). In wheat (*Triticum turgidum*), the senescence-associated NAC (for no apical meristem [NAM], Arabidopsis transcription activation factor [ATAF], and cup-shaped cotyledon [CUC]) transcription factor GRAIN PROTEIN CONTENT-B1 positively regulates the onset of leaf senescence as well as the translocation of zinc and iron to grains (Uauy et al., 2006). Also, the transition metal molybdenum, an essential cofactor of enzymes involved in N assimilation, sulfite detoxification, and phytohormone biosynthesis, is readily remobilized upon senescence (Bittner, 2014).

Considering the investment of plants in nutrient acquisition, the remobilization of macronutrients and micronutrients during senescence is critical for efficient nutrient usage and for plant survival. The onset of senescence is strictly regulated and occurs under optimal conditions in an age-dependent manner (Fig. 1). However, upon exposure to environmental stress or nutrient deficiency, the plant can execute the senescence program as an adaptive response to promote survival and reproduction.

In this review, we address the role of senescence as an adaptive strategy to help the plant respond to its fluctuating environment, and we also discuss the extent to which manipulating this process would be beneficial to agriculture. First, we focus on internal and external factors that determine the onset of senescence, and we highlight the importance of the senescence process during plant adaptation to environmental stress. Next, we discuss sink-source relations and the adaptive advantage of senescence for plant survival in the field. Finally, we explore the role of senescence in regulating crop yield and grain quality and its implications for agriculture.

ONSET OF LEAF SENESCENCE

Under optimal growth conditions, the onset of leaf senescence occurs in an age-dependent manner (Schippers et al., 2007). Leaf senescence involves a complex interplay between internal and external factors, which determine the timing, progression, and completion of senescence. The model plant species Arabidopsis exhibits two types of senescence: sequential and reproductive senescence. During sequential senescence, older leaves senesce and their nutrients are translocated to younger, growing parts of the plant. This type of senescence is independent of reproduction, since male and female sterility increase plant longevity, while the lifespan of individual leaves remains unaffected (Noodén and Penney, 2001). Reproductive senescence occurs at the whole-plant level in monocarpic plants (Fig. 1) and promotes seed viability and quality. First, we will introduce the concept of developmental senescence and the senescence window. We will then provide a concise overview of the role of plant hormones in the timing and progression of senescence.

Developmental Senescence and the Senescence Window Concept

The identification of molecular markers for leaf senescence was a great breakthrough, which paved the way for elucidating leaf senescence at the transcriptional level. For instance, age-dependent induction of senescence in leaves by ethylene was first demonstrated using *SENESCENCE ASSOCIATED GENE2* (*SAG2*) and *SAG12* as molecular markers (Grbić and Bleecker, 1995). The relationship between leaf age and ethylene-induced senescence was studied in detail by Jing et al. (2002), resulting in the concept of the senescence window (Fig. 2). Over time, the senescence window concept was extended and used to explain how the onset of senescence relies on the integration of hormones or external factors into leaf ageing (Schippers et al., 2007). The window concept assumes three distinct leaf developmental phases in relation to the induction of senescence. The first phase corresponds to early development (growth) and is a never-senescence phase. Leaves, which arise as heterotrophic cell outgrowths from the shoot apical meristem, act as sink tissues during their early phase of development. During the phase of proliferation and expansion, the leaf responds differently to senescence-inducing factors (Graham et al., 2012). For instance, ethylene application to growing leaves does not induce senescence, instead resulting in reduced cell proliferation and expansion (Skirycz et al., 2010). In other words, the strategy of the plant is to protect young tissues from precocious senescence. Maturation of the leaf represents the second phase of the senescence window concept, during which the leaf becomes competent for internal and external factors to activate senescence (Fig. 2). The effect of senescence-inducing factors at this stage increases with leaf age, indicating that the leaf becomes more competent to undergo senescence. In an attempt to explain this observation, the term ARCs was introduced (Jing et al., 2005; Schippers et al., 2007). During leaf development, these ARCs accumulate to a level at which senescence will be induced even under optimal growth conditions, as illustrated by the final phase of the senescence window concept (Fig. 2). However, although leaves become more permissive to the induction of senescence with age, they remain competent for perceiving senescence-delaying or -reverting signals (Gan and Amasino, 1995), indicating that the accumulation of ARCs does not affect the vigor of the leaf.

Ethylene

Ethylene induces a senescence program that has physiological, biochemical, and genetic features of developmental leaf senescence. Mutating ethylene signaling or biosynthesis genes affects the timing of senescence (Graham et al., 2012; Bennett et al., 2014).

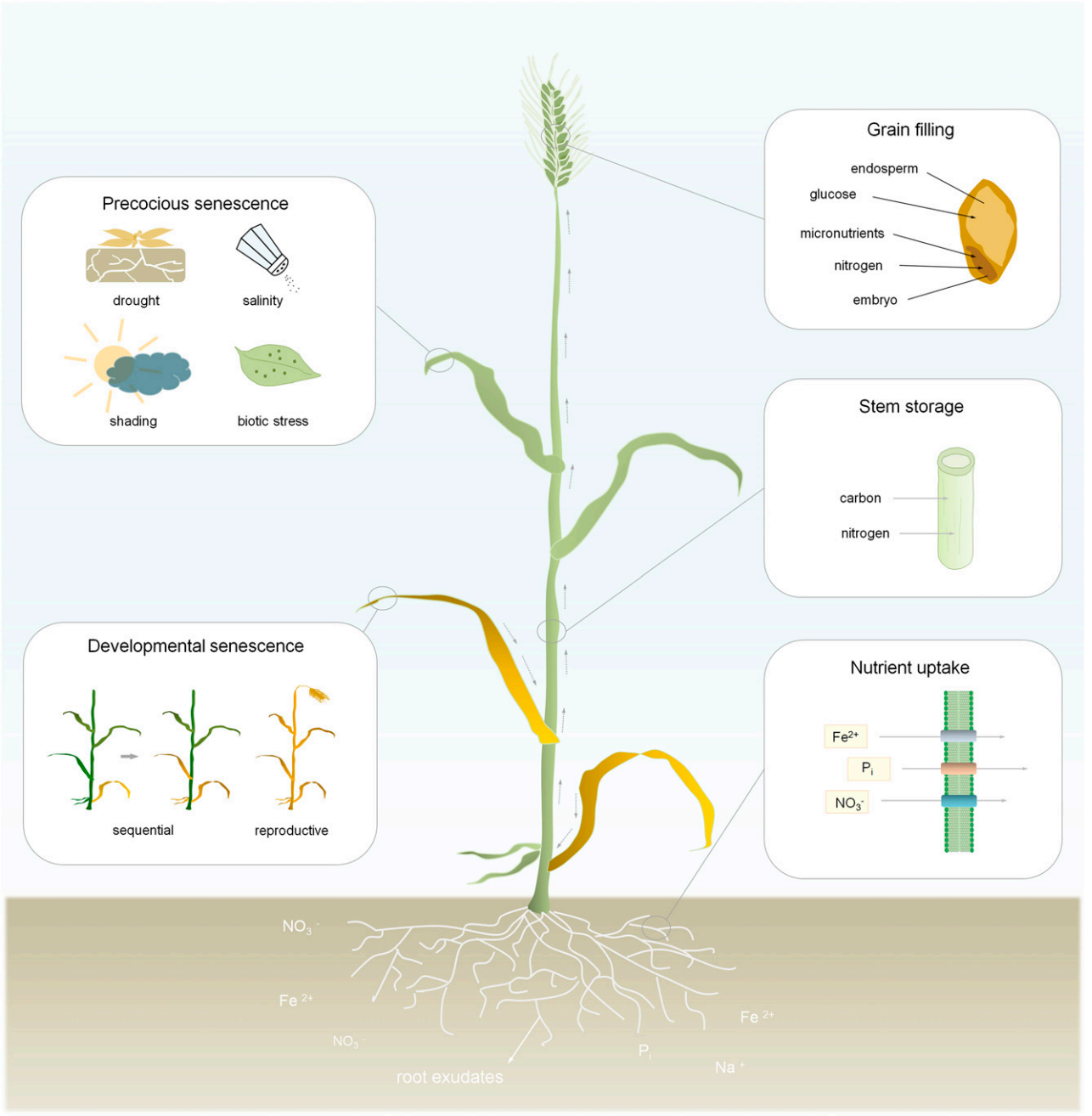

Figure 1. Overview of nutrient remobilization and transport during developmental and precocious senescence. Under optimal conditions, plants undergo developmental senescence. Two types of developmental senescence can occur. During sequential senescence, the nutrient salvage program begins with the oldest leaves and follows the age gradient within the plant. By contrast, reproductive senescence occurs at the whole-plant level in monocarpic plants and involves the nearly simultaneous dismantling of all leaves to support grain filling. However, under adverse environmental conditions, including shading, drought, salt, and biotic stress, the senescence program is initiated as part of the acclimation response. The uptake of nutrients from the soil is an energy-expensive process. Therefore, the salvaging of these nutrients during leaf senescence greatly contributes to the nutrient usage efficiency of the plant. During vegetative growth, large portions of photoassimilates and N-containing compounds are temporarily stored in stem tissues. These reserves are remobilized during whole-plant senescence. The formation of reproductive sink tissues greatly stimulates the onset of senescence in many plant species. In particular, carbon, N, and micronutrients are translocated to the developing seeds.

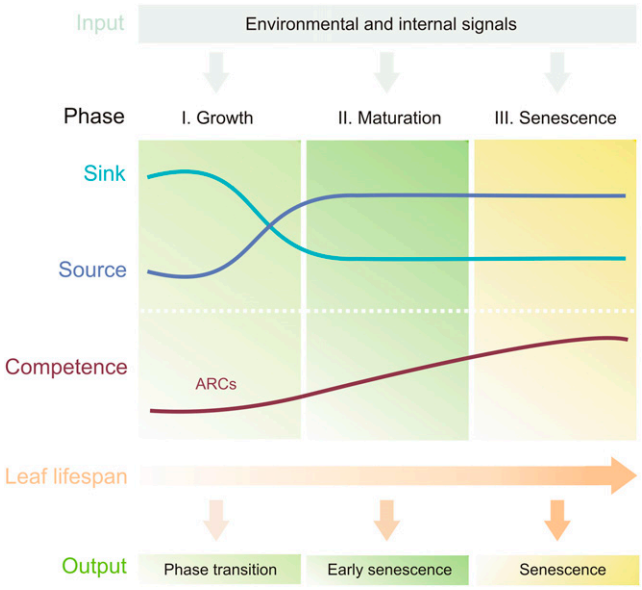

Figure 2. The senescence window concept. The lifespan of the leaf covers several developmental transitions, which are influenced by both internal and external signals. During the growth phase (I), the leaf undergoes a sink-to-source transition, and environmental stress signals do not induce senescence, but they interfere with the growth process. As an output, these signals cause an early transition to maturation of the leaf by affecting the processes of cell proliferation and expansion. Once a leaf reaches maturity (II), it becomes competent to undergo senescence. The competence to senesce increases with age, due to the accumulation of age-related changes (ARCs). As ARCs continue to accumulate, the leaf is more prone to senesce and will eventually undergo developmental senescence (III), irrespective of adverse environmental conditions.

For instance, the ethylene-insensitive mutants *ethylene receptor1-1* and *ethylene insensitive2* (*ein2*) exhibit delayed senescence (Grbić and Bleecker, 1995; Alonso et al., 1999), while overexpressing the transcription factor gene *EIN3* causes early leaf senescence (Li et al., 2013). Ethylene signaling relies on the nuclear translocation of EIN2 and the subsequent activation of two transcription factors, EIN3 and EIN3-LIKE1 (EIL1; Chang et al., 2013). Recently, an extensive genome-wide chromatin immunoprecipitation assay for EIN3 was performed, covering seven time points after ethylene treatment (Chang et al., 2013), which resulted in the identification of 1,314 candidate target genes of EIN3. Considering the role of ethylene in senescence, we compared the target list with genes known to be induced during senescence (Guo et al., 2004; Buchanan-Wollaston et al., 2005), finding that 269 *SAGs* are among the reported EIN3 targets (Fig. 3A; Supplemental Table S1), which we refer to as *EIN3-BOUND SAGs* (*EB-SAGs*). The study by Chang et al. (2013) was performed on seedlings, which (according to the senescence window) are in the never-senescence phase. Indeed, this simultaneous expression profiling revealed that only 76 of the 269 *EB-SAGs* are responsive to ethylene at the seedling stage. Thus, binding of EIN3 to an *EB-SAG* promoter is, in most cases, not sufficient to activate the senescence program, suggesting that an additional component is required.

As ethylene induces senescence in many plant species, we examined whether the transcriptional network downstream of EIN3 is conserved. To this end, we performed bidirectional BLAST searches with the Arabidopsis *EB-SAGs* against the rice (*Oryza sativa*) genome using the Phytozome database (Goodstein et al., 2012). Interestingly, we found rice homologs for 159 Arabidopsis *EB-SAGs*, and in more than 90% of the cases, at least one EIN3 core binding site (TACAT) was found in the upstream promoter regions (Supplemental Table S1). These findings suggest that ethylene controls similar processes during senescence in Arabidopsis and rice. Gene Ontology analysis (Proost et al., 2009) further revealed a significant enrichment for terms related to catalytic activity, transcription, and transport (Fig. 3B), which is in line with previous reports demonstrating that ethylene is required for nutrient remobilization during senescence (Jung et al., 2009).

Cytokinin

Richmond and Lang (1957) reported that cytokinin (CK) delays the onset of senescence by preventing chloroplast breakdown. The senescence-delaying feature of CK is commonly used by pathogens and herbivores to establish so-called green islands (Walters et al., 2008). By placing a CK biosynthesis gene encoding an isopentenyl transferase (IPT) under the control of the *SAG12* promoter, it is possible to retard developmentally induced senescence (Gan and Amasino, 1995). In addition, drought-induced senescence can be prevented by placing the *IPT* gene under the control of a stress- and maturation-induced promoter (Rivero et al.,

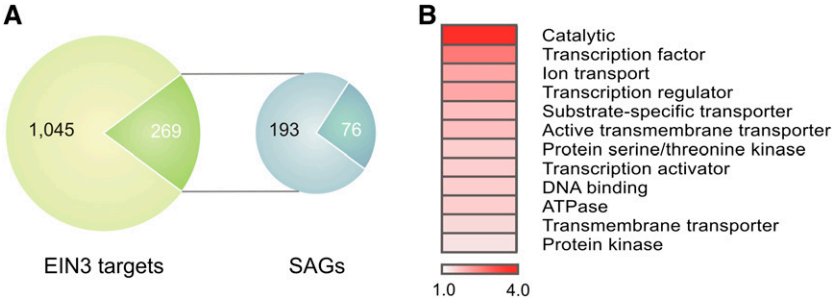

Figure 3. The EIN3 senescence-associated gene network. A, Among the direct targets of EIN3 are 269 SAGs (green), 76 of which are induced by ethylene during seedling establishment (dark blue). B, Gene Ontology enrichment analysis of EIN3-controlled *SAGs*, as determined by the PLAZA workbench (Proost et al., 2009). The results are given as a heat map in which the scale bar represents the $-\log_{10}$ of the *P* value. All listed categories were significantly enriched.

2007). The mechanisms behind CK-delayed senescence mainly involve metabolic reprogramming that assigns a sink signature to the organ. CK treatment results in the coordinated induction of an extracellular invertase (*CIN1*) and hexose transporter genes, leading to higher uptake of hexoses (Ehness and Roitsch 1997). Invertases mediate the hydrolytic cleavage of Suc into hexose monomers at the site of phloem unloading, and metabolization of these cleavage products controls the sink strength (Roitsch and González, 2004). Interestingly, in plants with reduced extracellular invertase activity, CK fails to delay senescence (Balibrea Lara et al., 2004). Moreover, placing *CIN1* under the control of the *SAG12* promoter results in delayed senescence, demonstrating that this gene acts downstream of CK. In addition, ARABIDOPSIS RESPONSE REGULATOR2 (ARR2), a positive regulator of CK responses, is a negative regulator of senescence, acting directly downstream of CK receptors (Kim et al., 2006). Whether ARR2 directly controls the activity of extracellular invertase remains to be tested. Taken together, these findings demonstrate that CK delays senescence by increasing the sink strength of the tissue.

Salicylic Acid

During the final phase of leaf development, salicylic acid (SA) levels increase (Breeze et al., 2011). Mutants defective in SA signaling, such as *phytoalexin deficient4* (*pad4*) and *nonexpressor of pathogenesis-related genes1* (*npr1*), exhibit altered *SAG* expression patterns and delayed senescence (Morris et al., 2000). In addition, *pad4* mutant leaves exhibit senescence symptoms but are largely nonnecrotic, supporting a role for SA in the transition from senescence to final cell death. Interestingly, SA was recently shown to play a dual role in promoting cell death and survival. Notably, the delayed-senescence phenotype of plants with reduced SA biosynthesis occurs at the expense of defense responses (Fu et al., 2012). NPR1, which functions as a central activator of SA responses, is targeted for proteasomal degradation by the SA receptors NPR3 and NPR4 (Fu et al., 2012). NPR3 and NPR4 can both bind to SA, but NPR4 has a much higher affinity for this phytohormone. Very high concentrations of SA cause rapid degradation of NPR1 through the action of NPR3, which is followed by programmed cell death. By contrast, NPR4 only targets NPR1 for degradation when it is not bound to SA. Thus, under basal SA levels, NPR1 is stabilized and can promote defense responses and plant survival. This process involves the accumulation of PATHOGENESIS RELATED (PR) proteins as well as endoplasmic reticulum stress responses that induce autophagy (Minina et al., 2014). During leaf development, SA accumulates in an age-related manner, resulting in the NPR1-dependent endoplasmic reticulum stress activation of autophagy in older tissues (Yoshimoto et al., 2009; Minina et al., 2014). Autophagy is a proteolytic process in eukaryotic cells involving the regulated breakdown of proteins and amino acid recycling via nonselective lysosomal/vacuolar proteolysis (Ono et al., 2013). During senescence, autophagy is important for N remobilization through its role in supporting the dismantling of the chloroplast (Fig. 4), which constitutes an essential aspect of the nutrient remobilization program (Guiboileau et al., 2012). In addition, autophagy plays an NPR1-dependent, protective role in promoting cell survival during cellular stress provoked by senescence. Indeed, the *autophagy5* mutant exhibits precocious senescence and cell death (Yoshimoto et al., 2009). Hence, autophagy operates as a negative feedback loop, modulating SA signaling and cell death to allow for efficient nutrient recycling during senescence.

Abscisic Acid

Senescing leaves are characterized by an increase in abscisic acid (ABA) levels (Breeze et al., 2011), which promotes chloroplast degradation and leads to impressive multicoloring of leaves upon carotenoid demasking. ABA plays a dual role by repressing chloroplast biosynthesis genes and inducing genes that promote chlorophyll degradation during senescence (Liang et al., 2014). This is in contrast to the role played by ABA during earlier plant development, when it has a positive effect on chloroplast development (Kim et al., 2009a), as well as its role in mature leaves, when it induces a very different set of genes from those that are induced during developmental leaf senescence in older tissues (Guo and Gan, 2012). Exogenous application of ABA to rice flag leaves results in reduced chlorophyll contents and increased remobilization of carbon reserves (Yang et al., 2002). The senescence-promoting effect of ABA has been linked to its role in sugar signaling (Pourtau et al., 2004). Exogenous sugars can induce senescence, and during ageing, sugars accumulate prior to the onset of leaf senescence (Wingler and Roitsch, 2008). Moreover, the Glc-insensitive *aba insensitive5-1* (*abi5-1*) and *hexokinase1* (*hxk1*) mutants display delayed onset of senescence (Moore et al., 2003; Pourtau et al., 2004). Again, several ABA-deficient mutants exhibit precocious senescence even though they are Glc insensitive, suggesting that ABA might not be required for sugar-induced leaf senescence (Pourtau et al., 2004). However, it should be noted that ABA deficiency impairs stomatal closure, resulting in drought hypersensitivity, which makes it difficult to untangle the relationship between Glc and ABA in regulating the onset of senescence.

ABI5 was recently found to directly regulate the expression of the NAC transcription factor gene *ORESARA1* (*ORE1*; Sakuraba et al., 2014), a regulator of developmental leaf senescence (Kim et al., 2009b). In addition, ABI5 controls the expression of *STAYGREEN1* and *NONYELLOW COLORING1*, which encode enzymes involved in chlorophyll degradation (Kusaba et al., 2007; Park et al.,

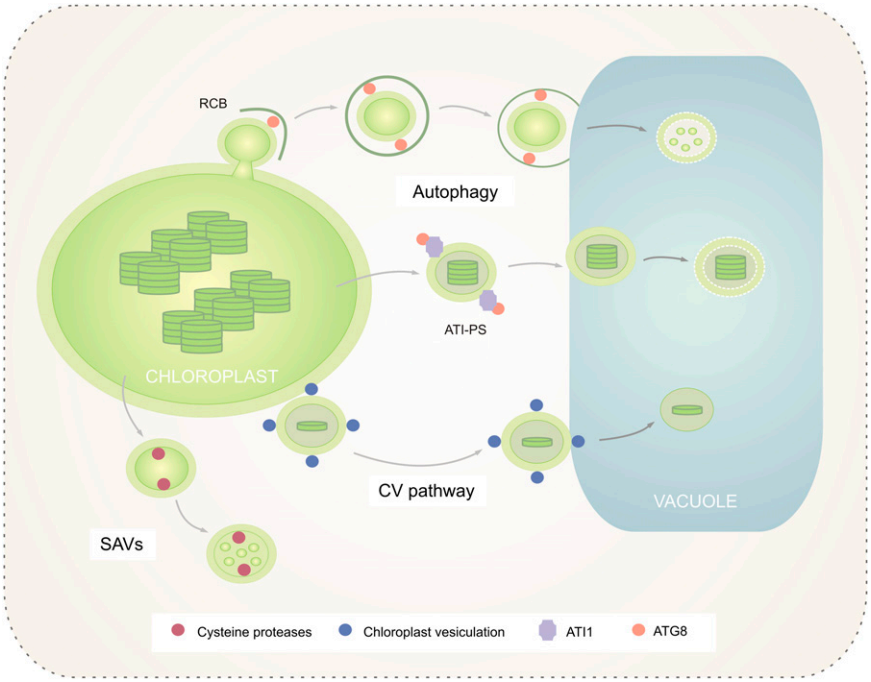

Figure 4. Chloroplast protein degradation pathways during senescence. Autophagic degradation of chloroplast proteins occurs through two specific pathways. The Rubisco-containing body (RCB) pathway begins with the formation of a chloroplastic protrusion, which becomes isolated and forms an autophagosome that is transported into the vacuole. Next, various autophagy-dependent plastid bodies (PS) containing ATG8-INTERACTING1 (ATI1) appear and specifically transport chloroplastic stromal proteins to the vacuole for degradation. In addition, there are two autophagy-independent pathways that regulate the degradation of chloroplastic proteins. First, chloroplast vesiculation (CV) promotes the formation of chloroplast protein-containing vesicles from chloroplast membranes and targets them to the vacuole for degradation. Alternatively, stromal proteins are translocated to senescence-associated vacuoles (SAVs), which contain Cys proteases and thus exhibit proteolytic activity. Hence, stromal proteins can be directly degraded in senescence-associated vacuoles instead of being transported to the central vacuole.

2007). Furthermore, the key senescence regulator NAC-LIKE, ACTIVATED BY APETALA3/PISTILLATA (NAP) in Arabidopsis directly activates the ABA biosynthesis gene *ABSCISIC ALDEHYDE OXIDASE3* (*AAO3*), thereby promoting ABA accumulation and senescence (Yang et al., 2014). In addition to promoting leaf senescence by inducing certain transcription factor genes, ABA also promotes leaf senescence via its effect on kinases. An ABA-activated mitogen-activated protein kinase cascade consisting of MAPKKK18, MKK3, and MPK1/2/7 positively regulates senescence in Arabidopsis (Danquah et al., 2015). ABA application increases the kinase activity of MAPKKK18, and Arabidopsis plants expressing a catalytically inactive variant of MAPKKK18 display a delayed-senescence phenotype (Matsuoka et al., 2015). Taken together, these findings suggest that ABA coordinates photosynthetic carbon metabolism with leaf age to positively regulate the onset of senescence and the breakdown of chlorophyll.

Jasmonates

Upon senescence, endogenous levels of jasmonic acid (JA) and the transcript levels of genes involved in JA biosynthesis and signaling increase (He et al., 2002). Among the *EB-SAGs* are those encoding *JASMONATE-ZIM-DOMAIN PROTEIN1* (*JAZ1*) and *JAZ6* (Supplemental Table S1), which act as negative regulators of JA signaling (Chico et al., 2008). TEOSINTE BRANCHED/CYCLOIDEA/PCF4 (TCP4) activates the JA biosynthesis gene *LIPOXYGENASE2* (*LOX2*), which promotes the onset of leaf senescence (Schommer et al., 2008). TCP transcription factors are mainly known for their role during early leaf growth, but recent studies support a link between TCPs, JA biosynthesis, and senescence (Danisman et al., 2012). The *tcp9* and *tcp20* mutants display precocious dark-induced senescence. Interestingly, TCP20 represses *LOX2* activity during early leaf growth to promote cell proliferation, thereby restricting JA accumulation, which acts as an inhibitor of cell proliferation (Pauwels et al., 2008). This function is opposite that of TCP4, indicating that JA homeostasis is controlled by TCP factors with contrasting functions. Indeed, class I and class II TCP factors bind directly to the promoter of *LOX2* to antagonistically regulate its expression (Danisman et al., 2012). During leaf ageing, the mRNA levels of class I TCP factors (repressing JA biosynthesis) decrease compared with those of class II TCP factors, eventually leading to increased JA biosynthesis during the onset of leaf senescence. This intriguing timing mechanism of antagonistic control over

JA biosynthesis might represent a type of internal clock that defines an important ARC that sets the age of the leaf.

Gibberellic Acid and Auxin

The transition from vegetative to reproductive growth is essential for reproductive success in plants. GA_3 induces flowering, thereby restricting the lifespan of monocarpic plants (Evans and Poethig, 1995). In line with this observation, application of bioactive GA_3 promotes reproduction and subsequent senescence in Arabidopsis (Chen et al., 2014). In the absence of GA, DELLAs repress the GA signaling pathway. Upon perception of GA by the GA receptor GIBBERELLIN INSENSITIVE DWARF1, proteasome-mediated degradation of DELLA proteins occurs (Ueguchi-Tanaka et al., 2005). The quintuple mutant *Q-DELLA*, which has lost the repressive effect of DELLAs, exhibits precocious developmental senescence. By contrast, knockout of the GA biosynthesis gene *GA REQUIRING1* (*GA1*) results in the accumulation of DELLA proteins as well as delayed development and onset of senescence (Chen et al., 2014). Therefore, GA may prolong the lifespan of individual leaves; however, by promoting reproductive development, it can also restrict the total lifespan of the plant.

The involvement of auxin in regulating leaf senescence is suggested by the presence of genes that are auxin responsive and encode AUXIN RESPONSE FACTORS (ARFs) or AUXIN/INDOLE ACETIC ACID (IAA) proteins. However, at the transcript level, many of these genes are not only regulated by auxin but also by other phytohormones (Audran-Delalande et al., 2012). Auxin is generally seen as a senescence-retarding compound, whose levels transiently increase (in the form of IAA) during the progression of senescence (Quirino et al., 1999). Auxin treatment effectively represses *SAG12* in senescing leaves (Noh and Amasino, 1999), implying that auxin functions in the maintenance of cell viability during senescence (Schippers et al., 2007). The molecular mechanism underlying the repressive effect of auxin on *SAG12* expression was recently demonstrated in the *wrky57* mutant, which displays early onset of senescence (Jiang et al., 2014). *WRKY57* is induced by auxin and acts as a direct repressor of *SAG12*. Interestingly, the effect of WRKY57 on *SAG12* expression is antagonized through its interaction with IAA29 (Jiang et al., 2014). The transcript abundance of *ARF2*, encoding a repressor of auxin responses, increases during senescence (Ellis et al., 2005). Loss-of-function mutation of *ARF2* results in dark green leaves, delayed flowering, and the onset of leaf senescence (Ellis et al., 2005). A mutant derived from a cross between *arf2* and *ein2* exhibits a further delay in senescence, suggesting that ARF2 acts independently of ethylene. Two additional senescence-related ARFs that are highly similar to ARF2, namely ARF7 and ARF19, additively regulate the onset of senescence with ARF2. The triple *arf2/arf7/arf19* mutant shows an enhanced late-senescence phenotype (Ellis et al., 2005). ARF2, ARF7, and ARF19 were recently found to repress the expression of two GA-implicated transcription factor genes, *GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*)/*CYTOKININ-RESPONSIVE GATA FACTOR1* (Richter et al., 2013). Overexpression of both *GNC* and *GNL* results in a delayed onset of senescence, while introducing *gnc* and *gnl* loss-of-function alleles into the *arf2* background suppresses the delayed-senescence phenotype of *arf2*. Interestingly, transcriptome profiles of plants overexpressing *GNC* or *GNL* largely resemble those observed for the delayed senescence mutant *ga1* (Richter et al., 2013). As *ARF2* is induced by GA, GA-auxin cross talk during senescence may occur via the following model: GA promotes the abundance of *ARF2* and thereby represses *GNC* and *GNL* transcription; the repression of *GNC* and *GNL* by ARF2 results in the activation of leaf senescence. Such a model could explain the observed effect of GA on the lifespan of the plant.

ENVIRONMENTALLY INDUCED SENESCENCE

During its lifetime, a plant is exposed to various environmental conditions that can prematurely induce the senescence program (Fig. 1). The primary response to stress is impaired growth, which generally results in assimilate accumulation in source leaves due to reduced sink activity, thereby triggering premature senescence (Albacete et al., 2014). Here, we provide a brief overview of the abiotic and biotic stresses that promote senescence.

Salt Stress

Salt stress harms the plant in two ways: the occurrence of osmotic stress leads to reduced cell turgor and inhibits leaf growth, and the accumulation of sodium ions (Na^+) is toxic (Munns and Tester, 2008). However, the primary factor might not be the buildup of Na^+ but rather the impaired sink strength and assimilate accumulation in source leaves (Albacete et al., 2014). That said, the accumulation of Na^+ in older leaves might promote the survival of young tissues to ensure reproductive success under salt stress. However, it remains to be demonstrated whether the remobilization of nutrients from salt-saturated leaves actually occurs. Indeed, two winter wheat cultivars differing in their ability to cope with salt stress exhibit opposite senescence induction patterns (Zheng et al., 2008). Specifically, while for the salt-sensitive cultivar, the duration of reproductive growth and total growth is reduced under various salt concentrations in a linear manner, the salt-tolerant cultivar remains unaffected. The delayed senescence in the tolerant cultivar during salt stress can be explained by an increase in sink strength (Zheng et al., 2008).

Overexpression of *SAG29* (*SWEET15*), a Suc transporter, causes early senescence and increased sensitivity

to salt stress (Seo et al., 2011). Notably, although *SAG29* transcripts strongly accumulate during senescence, a translational fusion protein is barely detectable in leaves undergoing senescence. On the other hand, SAG29 is present in developing seeds (Chen et al., 2015), indicating that SAG29 might control sink strength. Therefore, the early-senescence phenotype of *SAG29* overexpression plants can be explained by the interference of SAG29 with sink-source interactions (Chen et al., 2015). Consistent with this notion, overexpressing an apoplastic invertase gene (causing increased sink strength) results in improved salinity tolerance in *Nicotiana benthamiana* (Fukushima et al., 2001). Thus, delayed senescence and increased sink strength of the growing parts of the plant can contribute to salinity tolerance.

Senescence-related leaf parameters, such as chlorophyll content, protein content, and lipid oxidation, are greatly affected in tomato (*Solanum lycopersicum*) plants exposed to salt stress (Ghanem et al., 2008). Notably, salt stress stimulates ABA and 1-aminocyclopropane-1-carboxylic acid (ethylene precursor) accumulation but results in a decline in IAA and total CK contents. However, only 1-aminocyclopropane-1-carboxylic acid promotes senescence under salt stress, as its accumulation has been linked to both the onset of oxidative stress and the decline in chlorophyll fluorescence, while changes in the concentrations of IAA, CK, and ABA appear to play only minor roles in the regulation of salt-induced senescence (Ghanem et al., 2008).

Drought Stress

Drought stress represents a major threat to crop productivity worldwide (Cramer et al., 2011). Like soil salinity, water deprivation leads to osmotic stress, which impairs plant growth. During reproductive senescence, cereal crops exhibit carbon reserve remobilization, which enables the translocation of preanthesis assimilates from leaves and stems to the developing grain (Gregersen et al., 2013). Under ideal conditions (i.e. sufficient water availability), the contribution of carbon reserves stored in wheat stems to final grain weight is lower than that in plants under drought stress (Schnyder, 1993). Drought-induced senescence might compensate for the shorter grain-filling phase and lower photosynthetic activity observed under stress (Yang et al., 2002). A comparative proteomics study of wheat landraces exposed to postanthesis drought stress revealed that proteins involved in leaf senescence contribute to the remobilization of stem-derived carbohydrates (Bazargani et al., 2011). It appears that drought stress redirects the biosynthesis of stem-specific proteins and stimulates both stem senescence and reserve remobilization to compensate for the lower rates of assimilate synthesis (Bazargani et al., 2011).

Water deprivation in rice causes a rapid increase in the level of ABA in flag leaves, while CK levels gradually decline (Yang et al., 2002). Manipulating CK levels leads to a delay in drought-induced senescence. *IPT* expression driven by a stress- and maturation-inducible promoter further improves drought tolerance in tobacco (*Nicotiana tabacum*; Rivero et al., 2007), maintaining a seed yield similar to that of well-watered plants. Taken together, these findings suggest that modifying the expression of target genes involved in CK biosynthesis represents a promising breeding strategy for enhancing drought stress tolerance by delaying senescence.

Dark-Induced Senescence

The effect of light (or the lack of it) on the induction of senescence is multifaceted, as this effect largely depends on both the intensity and the type of light. In principle, light intensities either above or below the optimal level can cause premature senescence (Lers, 2007). The transcription factor SUBMERGENCE1A (SUB1A), a key regulator of submergence in rice, increases tolerance to dark-induced senescence (Fukao et al., 2012). The characteristic loss of chlorophyll and carbon reserves in photosynthetic tissues upon light deprivation is much less prominent in *SUB1A* overexpression plants than in the wild type. As a consequence, the recovery of photosynthetic activity after incubation in darkness is enhanced in these plants (Fukao et al., 2012). The protective role of SUB1A against dark-induced senescence is achieved through the repression of an ethylene response pathway. Interestingly, in rice, ethylene promotes growth to allow plants to escape from submergence, which, in turn, is repressed by SUB1A. Therefore, the increased tolerance to darkness provided by SUB1A might (in part) represent an energy-saving strategy.

Recently, the molecular mechanism underlying dark-induced senescence was uncovered in Arabidopsis (Sakuraba et al., 2014). In the absence of light, *PHYTOCHROME INTERACTING FACTOR4* (*PIF4*) and *PIF5* mRNA and protein accumulate, resulting in the activation of several downstream transcriptional regulators, including ORE1, ABI5, and EIN3. The activation of these positive regulators of leaf senescence causes a robust initiation of the senescence program at the transcriptional level, which helps dismantle the leaf. The expression of *SAGs* during dark-induced senescence relies on intact JA and ethylene but not on SA signaling (Buchanan-Wollaston et al., 2005). In line with this observation, the ethylene signaling genes *EIN2* and *EIL1* and the JA signaling genes *JAZ1* and *MYC2* act downstream of PIF4 (Oh et al., 2012), while SA genes such as *NPR1* and *NIM1-INTERACTING1* do not. These findings indicate that dark-induced senescence is a tightly regulated process and that PIF4/PIF5 may coordinate the activation of senescence regulators under such stimulation.

Nutrient Limitation

Plants require both macronutrients and micronutrients in order to successfully complete their life cycle.

Not unexpectedly, plants are often faced with a variable amount of nutrients in their environment, which (under extreme circumstances) can cause starvation (Fischer, 2007). In response to nutrient limitation, plants initiate leaf senescence to promote nutrient recycling and mobilization.

Under N-limiting conditions, senescence is induced to remobilize N via chloroplast dismantling (Masclaux et al., 2000). Degradation of the chloroplast relies in part on autophagy (Fig. 4), a bulk degradation mechanism that targets cytoplasm and organelles for vacuolar breakdown (Ono et al., 2013). Autophagy of the chloroplast involves the delivery of two types of autophagic bodies to the vacuole, Rubisco-containing bodies (Chiba et al., 2003; Ishida et al., 2008) and ATI1 plastid bodies (Michaeli et al., 2014), both of which contain stroma proteins. Arabidopsis autophagy mutants are characterized by impaired N remobilization, but they can still complete their life cycle. In addition, an autophagy-independent pathway for delivering chloroplast proteins to the vacuole was recently discovered. These delivering bodies contain a so-called CHLOROPLAST VESICULATION protein, which is especially found upon exposure to stress and serves to dismantle the chloroplast (Wang and Blumwald, 2014). Not all chloroplast proteins are degraded in the vacuole. During senescence, proteolytically active small senescence-associated vacuoles accumulate and degrade soluble photosynthetic proteins (Otegui et al., 2005).

Sulfur (S) is an essential macroelement for crops, whose deprivation and remobilization mainly depend on the N status of the plant (Fischer, 2007). In contrast to N limitation, S deficiency can induce the recycling of stored S in leaves without any acceleration of leaf senescence symptoms (Dubousset et al., 2009). However, under both low-N and low-S conditions, proteins are also salvaged to retrieve stored S for remobilization.

Grasses secrete phytosiderophores, which chelate Fe (III) from their roots to the rhizosphere to enhance iron acquisition (Itai et al., 2013). Iron deficiency in barley (*Hordeum vulgare*) specifically causes senescence of the oldest leaf (Higuchi et al., 2014). ^{13}C-tracer experiments revealed the preferential allocation of assimilates from the senescing leaf to the roots to enable phytosiderophore secretion. Thus, upon exposure to nutrient-limiting conditions, premature senescence of a single leaf can promote whole-plant survival.

Biotic Stress

Pathogens and herbivores strongly affect crop production and can threaten plant survival. Their attack causes either rapid or prolonged reactions in the plant in the form of defense responses or disease syndromes, which in diverse ways can lead to the acceleration of senescence (Gregersen et al., 2013). The transcriptional program that operates upon biotic stress largely overlaps with that during developmental senescence (Guo and Gan, 2012).

With age, Arabidopsis becomes resistant to virulent *Pseudomonas syringae* pv *tomato*, a defense response known as age-related resistance (Kus et al., 2002). Interestingly, the delayed senescence mutants *ein2*, *ore1*, and *nac055* exhibit an impaired onset of age-related resistance (Al-Daoud and Cameron, 2011), suggesting that an increased commitment to senescence improves plant resistance against *P. syringae* pv *tomato*. The necrotrophic fungal pathogen *Botrytis cinerea* has a broad host range and causes both preharvest and postharvest diseases (Windram et al., 2012). Numerous genes up-regulated during *B. cinerea* infection are genuine *SAGs* (Guo and Gan, 2012; Windram et al., 2012). In both cases, genes involved in photosynthesis, chlorophyll biosynthesis, and starch metabolism are down-regulated under *B. cinerea* infection, while a large fraction of genes acting downstream of ethylene, ABA, or SA signaling are up-regulated. The expressional dynamics during *B. cinerea* infection occur in a much shorter time frame than those during senescence, implying that, to protect the plant, *B. cinerea*-infected cells undergo programmed cell death more rapidly, limiting the time available for nutrient recovery during pathogen attack.

During infection of Arabidopsis with the *Tobacco rattle virus* (TRV), a large overlap with the senescence program at the transcriptional level was also observed (Fernández-Calvino et al., 2015). In particular, the up-regulation of dark-inducible genes (DINs), including *DIN1*, *DIN6*, and *DIN11*, was observed in TRV-infected tobacco leaves. Moreover, knockdown of *DIN6* or *DIN11* reduced the susceptibility of tobacco and Arabidopsis to TRV. During the early infection phase, no visual senescence symptoms were observed, suggesting that the virus somehow uses the senescence program to its own benefit. Indeed, knockdown of *DIN11* impairs the in planta replication of TRV. Also, other virus infections in plants result in the activation of *SAGs* (Espinoza et al., 2007). However, it remains to be determined whether this represents a coordinated plant response or a provoked viral response.

MOLECULAR REGULATION OF SENESCENCE

Transcriptional Networks

During the onset and progression of senescence, several thousand genes are differentially expressed (Guo et al., 2004; Breeze et al., 2011). In recent years, small gene-regulatory networks for senescence-associated transcription factors have been uncovered (Schippers, 2015). Here, we consider three of these, NAP, WRKY53, and ORE1, and for simplicity, we focus on linear networks controlled by each factor in relation to a specific phytohormone.

Transfer DNA insertion lines for *NAP* exhibit a delayed onset of developmental senescence but normal progression of plant development and flowering (Guo and Gan, 2006), while overexpression of *NAP* causes precocious senescence. NAP activates the expression of

SAG113, encoding a PROTEIN PHOSPHATASE2C protein (Zhang and Gan, 2012). SAG113 negatively regulates ABA-mediated stomatal closure in order to promote water loss in leaves during senescence (Zhang et al., 2012). In addition, in ABA signaling mutants, *SAG113* expression during senescence is impaired, indicating that this gene acts downstream of the ABA signaling cascade. The relationship between ABA and NAP is rather complex, as NAP promotes ABA biosynthesis during the onset of senescence by positively regulating the expression of *AAO3*, which is responsible for the final step in ABA biosynthesis (Yang et al., 2014). Consequently, *nap* mutants fail to accumulate ABA during senescence. Exogenous application of ABA on *nap* leaves and constitutive overexpression of *AAO3* in the *nap* mutant restore senescence progression (Yang et al., 2014). Interestingly, the function of NAP in the regulation of ABA-mediated senescence is conserved in crops. Like its Arabidopsis homolog, OsNAP in rice positively regulates leaf senescence in an ABA-dependent manner. In vitro and in vivo binding studies showed that OsNAP directly induces the expression of genes involved in chloroplast degradation and nutrient transport. While *OsNAP* overexpression plants have an early-senescence phenotype, knockdown of *OsNAP* results in a significant delay in leaf senescence. Notably, this late-senescing phenotype is accompanied by a prolonged grain-filling phase and an increased grain yield (of up to 10%) in *OsNAP* RNA interference lines (Liang et al., 2014).

WRKY53 represents another positive regulator of leaf senescence, which activates several senescence-related genes, including *SAG12*, *SAG101*, *CATALASE1/2/3*, and *ORE9* (Miao et al., 2004). *WRKY53* expression is induced by treatment with hydrogen peroxide, which correlates with the observed increased expression of *WRKY53* at the time of bolting, during which an increase in endogenous hydrogen peroxide levels has been reported (Miao et al., 2004). Furthermore, WHIRLY1 (WHY1), a protein implicated in plant defense, acts as a negative regulator of *WRKY53* expression (Miao et al., 2013). Interestingly, WHY1 was recently proposed to be a redox sensor that moves from the chloroplasts to the nucleus (Foyer et al., 2014). In addition, both WHY1 and WRKY53 are downstream components of SA signaling pathways that act independently of NPR1 (Desveaux et al., 2004; Miao and Zentgraf, 2007). The dismantling of chloroplasts during senescence may release WHY1 protein, which may (in part) suppress the action of WRKY53 to control the progression of senescence. Notably, the action of WHY1 is not limited to its control over *WRKY53*; additional genes, including PR genes, are modulated by WHY1 (Desveaux et al., 2004). Another redox sensor, the homeodomain Leu zipper transcription factor REVOLUTA (REV), positively regulates the expression of *WRKY53*. REV is mainly known for its role in setting organ polarity during early leaf development (Xie et al., 2014). Loss of *REV* results in a delayed onset of leaf senescence and attenuated induction of *WRKY53* expression upon hydrogen peroxide treatment. The connection between WRKY53 and REV suggests that early developmental processes may influence the ageing process and the subsequent onset of leaf senescence.

In conjunction with the above observation, *ORE1* expression gradually increases during leaf development (Kim et al., 2009b). *ORE1* transcript accumulation is regulated by the activity of *microRNA164* (*miR164*) in an ethylene-dependent manner (Kim et al., 2009b). Ethylene production gradually increases during leaf ageing, while *miR164* expression declines, allowing the accumulation/translation of *ORE1* transcripts. Moreover, EIN3 directly binds to the promoter of *miR164* to repress its expression, and this binding activity progressively increases during leaf ageing (Li et al., 2013). As *ORE1* itself is also a target of EIN3, it is highly likely that ethylene stimulates *ORE1* expression in a dual manner: on the one hand, ethylene represses *miR164* expression, while on the other, it directly activates *ORE1* expression. Consistent with this hypothesis, even a transient induction of *EIN3* is sufficient to accelerate senescence progression (Li et al., 2013). Like WRKY53, which functions upstream of other WRKY transcription factors, ORE1 functions upstream of a large set of senescence-related NAC transcription factors (Kim et al., 2014). In addition to ethylene-induced regulation of senescence by ORE1, ORE1 was recently found to act downstream of phyB-mediated light signaling to promote senescence under light-deprived conditions (Sakuraba et al., 2014).

Protein Degradation

Protein degradation occurs through the action of proteases and via the ubiquitin-proteasome system. At least a portion of senescence-associated proteases localize to senescence-associated vacuoles to degrade chloroplast-derived proteins (Carrión et al., 2013). While the proteasome can be found in the nucleus and cytosol, proteases localize to multiple cellular compartments, including the vacuole, chloroplast, and mitochondrion, as well as the secretory pathway. We will restrict our discussion to the role of ubiquitin-mediated protein degradation during senescence: in contrast to bulk degradation systems, this system can specifically target single regulatory proteins.

Ubiquitin-mediated degradation of proteins occurs throughout the life cycle of the leaf. Genes encoding proteasomal subunits exhibit relatively stable expression throughout leaf development (Kurepa and Smalle, 2008), suggesting that the capacity for protein degradation by the 26S proteasome is constant during ageing. This finding is not surprising, since targeted degradation by the proteasome is regulated through highly specific substrate recognition and ubiquitination involving approximately 1,500 E3 ligases (Vierstra, 2012). ORE9, an E3 ligase of the F-box family, restricts leaf longevity (Woo et al., 2001). ORE9 was subsequently identified as MORE AXILLARY GROWTH LOCUS2 (MAX2; Stirnberg et al., 2002), a central regulator of

lateral organ branching and strigolactone signaling. Interestingly, ORE9/MAX2 targets the brassinosteroid (BR) transcription factors BRASSINAZOLE RESISTANT1 (BZR1) and BZR2/BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 (BES1) for degradation (Wang et al., 2013). BR suppresses the expression of a large set of senescence-related NAC transcription factor genes, including *ATAF1*, *ANAC019*, *ANAC055*, and *ANAC072* (Chung et al., 2014), which might explain why the *ore9* mutant possesses a delayed-senescence phenotype. This notion is further supported by the observation that the *bes1* mutant exhibits early leaf senescence (Yin et al., 2002).

In turn, the senescence-induced RING E3 ligase RING-H2 FINGER A2A interacts with ANAC019 and ANAC055, which potentially limits their protein levels during senescence (Bu et al., 2009). The HECT domain E3 ligase UBIQUITIN PROTEIN LIGASE5 is required for the degradation of WRKY53, thereby repressing the onset of leaf senescence (Miao and Zentgraf, 2010). Moreover, the N-end rule pathway, a proteolytic branch of the ubiquitin system, has a major impact on the timing of senescence. The *delayed leaf senescence1* mutant harbors a transfer DNA insertion in *ARGININE-TRNA PROTEIN TRANSFERASE1* (*ATE1*), which tags target proteins containing a Cys, Asp, or Glu at their N termini for degradation (Yoshida et al., 2002). In line with this observation, the E3 ligase PROTEOLYSIS6, which functions downstream of ATE1, negatively regulates the onset of leaf senescence (Mendiondo et al., 2015). Furthermore, N-end rule components modulate early leaf development by limiting KNOX activity (Graciet et al., 2009). KNOXs activate CK biosynthesis, which might (in part) explain why a delayed-senescence phenotype is observed in N-end rule mutants. Thus, both specific targets of the ubiquitin-26S proteasome system and an entire branch of the pathway control the onset of senescence. As the Arabidopsis genome encodes nearly as many E3 ligases as transcription factors, the ubiquitin-mediated regulation of senescence is expected to be far more extensive than has been described to date.

SOURCE-SINK RELATIONSHIP AND SENESCENCE

Sink tissues are net importers of nutrients and assimilates, while source tissues supply the precursors for sink metabolism (Thomas, 2013). Assimilates are moved from source tissues to sinks through the vascular tissue, which also enables source-sink communication, thereby regulating the extent of assimilate movement. The relationship between source and sink organs in a plant changes during development and varies between plants with different reproductive strategies. Importantly, crop domestication has influenced the source-sink characteristics of crops in order to maximize their harvest index (Bennett et al., 2012). Crops execute senescence in a highly coordinated manner at both the whole-plant and organ levels. By contrast, the coordination of senescence across the whole plant is often quite poor in weedy species, thereby ensuring that, by releasing seeds over a protracted period, at least some of the seeds will be exposed to an environment that is favorable for germination.

Carbon-Nitrogen Resource Allocation

In principle, during its life cycle, the leaf undergoes a transition from a sink to a source organ, which occurs once it becomes photosynthetically active (Fig. 2). At maturity, the leaf provides carbon to the plant, while the initiation of senescence causes the leaf to become an N source until the death of the organ (Thomas and Ougham, 2014). The development of cereals is highly coordinated such that entire monocultures can be harvested on the same day, and even grains within the same ear mature over a narrow window. The flag leaf is the major contributor of carbon to cereals via canopy photosynthesis. This carbon source is used for starch production in developing grains, which is followed by a late influx of N mobilized from senescing vegetative tissues (Osaki et al., 1991).

Unlike cereals, which have a long history of domestication, oilseed rape (*Brassica napus*) and Arabidopsis still show considerable variation in N remobilization efficiency across related populations (Chardon et al., 2014; Girondé et al., 2015). Moreover, in many *Brassica* spp. crops, the photosynthetic stem and pod walls provide nutrients for developing seeds (Malagoli et al., 2005), reducing the requirement for leaf N remobilization during seed production (Wagstaff et al., 2009). Indeed, the stems of *B. napus* appear to act as transient storage organs when there is a mismatch between N demand by the seeds and the degree of leaf N remobilization (Girondé et al., 2015), which may be a consequence of the weedy traits that remain within leafy *Brassica* spp. crops.

Maize (*Zea mays*) breeding has altered how N in the developing grain is sourced. Remobilized N, an important contributor throughout plant growth, is derived from N taken up by the plant during the vegetative period. However, modern maize varieties also utilize N taken up by the plant during the reproductive phase, which is transported directly to the grain (Ciampitti and Vyn, 2013).

Source-Sink Communication

Successful reproduction in plants relies on fulfilling the sink demand for nutrients. Therefore, the flow of information between source and sink tissues is required to adjust the remobilization rate of nutrients. Weak sink strength would, in theory, cause a slower progression of senescence than strong sink strength. This is true in some cases, for instance in tobacco (Zavaleta-Mancera et al., 1999), while in several cereal species, this rule does not apply (Thomas, 2013).

Recent evidence suggests that sugar signaling plays a pivotal role in source-sink communication (Lin et al., 2014). The protein kinase SUCROSE NON-FERMENTING1-RELATED KINASE1 (SnRK1) is activated upon exposure to darkness and nutrient starvation, conditions that induce senescence. Interestingly, SnRK1-dependent sugar-demand signaling is necessary and sufficient for promoting the movement of the carbon supply from source tissues to growing/developing sinks (Lin et al., 2014). This observation suggests that sink demand controls nutrient remobilization from source tissues. In addition, environmental stresses counteract SnRK1 activity, reducing the sink strength, which is correlated with a reduction in growth. The lack of Glc sensing results in the delayed onset of senescence, as observed in the *hxk1* mutant (Moore et al., 2003), suggesting that this defect disrupts source-sink communication. On the other hand, the sink strength of seeds for N must also be satisfied by source tissues. In particular, grains with high storage protein biosynthesis have a massive demand for N (Kohl et al., 2012), but it is currently unclear how this demand is communicated between sink and source tissue.

ADAPTIVE ADVANTAGE OF LEAF SENESCENCE

The molecular processes underlying leaf senescence are strongly conserved between plant species, suggesting that senescence has evolved as a selectable trait in plants. The phenomenon of senescence is often portrayed as a paradox, as this trait promotes the death of the individual (Roach, 2003; Pujol et al., 2014). However, this view is too simplistic, as plants are not slated to die before they undergo successful reproduction. That said, plants are rather unusual organisms, as they can set their own lifespan according to environmental conditions, even before the viability and integrity of the plant are affected by degenerative ageing processes (Thomas, 2013).

Plants display continuous growth, which is a necessary consequence of being sessile. While the plant is growing and branching, its parts can encounter various environmental conditions that differ in terms of the availability of resources (Oborny and Englert, 2012). In particular, the root system utilizes a sophisticated foraging strategy to find novel nutrient resources once those in the immediate vicinity become depleted. To support root foraging, it is sometimes essential to recycle leaves to sustain root growth (Guan et al., 2014; Higuchi et al., 2014). Under both agricultural and natural field conditions, plants grow in dense stands, where they must compete for resources. For example, shading of leaves by neighboring plants reduces the photosynthetic efficiency and resource acquisition of the plant. The disposal of leaves that become inefficient due to neighbor competition allows for the rapid establishment of leaves at better positions in the canopy. The stay-green trait delays leaf senescence in many plant species (Thomas and Ougham, 2014), but this trait is actually undesirable when plants must compete for resources. For example, stay-green maize lines do not outcompete early-senescing lines when grown at high plant density (Antonietta et al., 2014). Therefore, senescence is essential for sustaining the phenotypic plasticity of growth, and it represents an important evolutionary trait that enables plants to adapt to the environment.

Although senescence occurs in an age-dependent manner in plants, ageing does not always involve a decline in viability (Thomas, 2013). As stated above, ageing in relation to development, including senescence, is best described using the definition of ARC, which refers to changes that occur during the time-based processes of growth and development. In the sense of morphological plasticity, the establishment of competence to senesce is an important ARC that allows the plant to respond adequately to adverse environmental factors. While the priority of young tissues is their own development, mature tissues operate for the benefit of the whole plant.

Agricultural practices, which date back more than 10,000 years, are dedicated to the careful selection of traits, including those that reduce branching/tillering and increase reproductive sink strength (Ross-Ibarra et al., 2007). As indicated above, the domestication process has strongly affected the coordinated execution of senescence. The uptake of nutrients from the soil ceases in *Brassica* spp. grown on fertile soil at the time of the floral transition, and nutrients required to complete the life cycle are derived from remobilization and pod photosynthesis. However, under nutrient-limiting conditions, *Brassica* spp. will continue to take up nutrients from the soil throughout the reproductive cycle (Rathke et al., 2006). This flexible strategy provides the plant with increased resilience to a range of environmental conditions, but unfortunately, the selection pressure for this degree of resilience has been lost through the selection of domesticated plants, which are usually grown under high-nutrient conditions. However, the rising demands for food production will require plants to be cultivated on more marginal lands or in areas in which abiotic environmental factors are suboptimal, in order to address food security. This might require the senescence process in current crops to be manipulated to make them suitable for agricultural use under suboptimal growth conditions. Manipulating the crop cycle could be equally important, such as enabling faster cropping during changing seasons or, alternatively, producing plants with longer establishment periods to allow them to capture more input from the environment.

IMPACT ON CROP YIELD AND FOOD QUALITY

From an agronomical perspective, senescence processes are immensely important, since most annual crop plants undergo reproductive senescence. In several cases, functional stay-green cultivars have been shown to possess enhanced crop yield (Gregersen et al.,

2013). However, the timing and efficiency of nutrient remobilization in crops are not only linked to yield, but they also strongly influence the nutritional quality of our food.

Reproductive Senescence and Crop Productivity

There is a close association between senescence of the flag leaf and induction of the seed maturation process in cereal crops (Kohl et al., 2012; Hollmann et al., 2014). Crop yield, as measured by grain number and weight, largely depends on the amount of assimilates that were captured and stored during the vegetative stage as well as the onset of the senescence process itself (Thomas and Ougham, 2014). In general, delayed senescence is thought to allow for prolonged assimilate capturing, which would improve crop productivity. Total grain yield in cereal species is determined by multiple components, including the number of spikes/panicles per plant, spike/panicle size, number of developing spikelets/grains per spike/panicle, and grain weight. Importantly, monocarpic senescence predominantly influences grain weight and, to some extent, grain number, while the other yield parameters are set before the initiation of reproductive senescence (Distelfeld et al., 2014). In rice, loss of *OsNAP* results in the delayed onset of senescence and a concomitant 6% to 10% increase in grain yield in the field (Liang et al., 2014). However, in *Triticum aestivum*, overexpression of *TaNAC-S* delays leaf senescence, resulting in an increased N content in the grain, but it has no effect on total yields (Zhao et al., 2015), indicating that delayed senescence does not always improve productivity. In a field experiment using four different maize lines displaying altered onset of leaf senescence, grain yields were similar, but N contents were lower, under nonstress conditions (Acciaresi et al., 2014). These results indicate that nutrient storage during the vegetative phase does not often limit the final yield of the plant. To increase crop yield, therefore, it is necessary to increase the sink capacity, which must be balanced by source remobilization of nutrients.

Senescence and Grain Quality

As stated above, delayed senescence is not always an effective strategy for increasing yield. In addition, many late-senescing phenotypes are actually representative of a delay in the entire life cycle, including the onset of N remobilization (Diaz et al., 2008). Hence, delayed senescence may negatively affect nutrient remobilization and reduce grain protein concentrations, thereby reducing the nutritional quality of our food.

Indeed, while delayed senescence can result in higher yields and biomass, the seeds contain a lower proportion of protein (Masclaux-Daubresse and Chardon, 2011). In many *Brassica* spp. crops, there is a negative correlation between seed N concentration and yield (Chardon et al., 2014). Also, in cereals, a negative correlation exists between protein concentration in the grain and plant yield, along with a delayed onset of senescence (Oury and Godin, 2007; Bogard et al., 2010; Blanco et al., 2012). On the other hand, a number of approaches have been taken to identify breeding lines with increased grain protein content but without reduced yields (Uauy et al., 2006; Jukanti and Fischer, 2008). In all cases, canopy senescence actually occurs more rapidly in these plants than in control lines. In addition, rapid senescence in *T. aestivum* has also been linked to an increase in the content of minerals such as iron and zinc in the grain, thereby improving the nutritional value (Uauy et al., 2006). Therefore, when breeding for early or delayed senescence, it is important to consider not only yield but also the nutritional value of the grain.

FUTURE PERSPECTIVES

Due to the growing world population and recent climate change, the development of more productive crops has become a central challenge for this century. The impact of senescence on crop yield and quality, and its potential use in breeding more environmentally resilient plants, are becoming increasingly important. In addition, adequate remobilization of nutrients increases the plant's nutrient usage efficiency, thereby reducing the requirement for fertilizers.

During the past decades, significant advances have been made in our understanding of the process of leaf senescence and its underlying regulation at the molecular level. In addition, a theoretical model (the senescence window concept) has emerged that explains how the competence to senesce is established during leaf development and how internal and external factors are integrated with age to define the timing of senescence. Furthermore, much of the fundamental knowledge of the regulation of senescence has been tested in crop species for its potential use in improving yield. This includes the stay-green traits (Thomas and Ougham, 2014) as well as *pSAG12:IPT* technology (Gan and Amasino, 1995). Further elucidating the senescence window, and the switch that renders plants competent to senesce, will enable the development of more focused strategies for manipulating senescence by targeting specific phases of development. Importantly, although a delay in senescence can have positive effects on the productivity of plants, these effects appear to largely depend on the plant species, environmental conditions, and yield parameters analyzed. In particular, the grain N content appears to be negatively affected by delayed senescence. Numerous researchers have discovered that trying to uncouple senescence regulatory pathways from stress responses is extremely difficult, since the genetic program underlying senescence largely overlaps with that of plant defense. Therefore, altering one senescence parameter might also reduce plant tolerance to stress.

There are still many unknowns in the complex relationship between senescence and crop productivity and

quality. However, the examples discussed in this review clearly demonstrate the potential of altering senescence in future breeding strategies. To this end, an integrative research effort is required, which not only focuses on the role of single genes in the onset of senescence but also examines conditions during which manipulation of the senescence process is beneficial to crop productivity and nutritional value.

Supplemental Data

The following supplemental materials are available.

Supplemental Table S1. *SAGs* that are direct targets of EIN3.

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