



## **Autophagy, plant senescence, and nutrient recycling**

Liliana Avila Ospina, Michaël Moison, Koki Yoshimoto, Celine  
Masclaux-Daubresse

### **► To cite this version:**

Liliana Avila Ospina, Michaël Moison, Koki Yoshimoto, Celine Masclaux-Daubresse. Autophagy, plant senescence, and nutrient recycling. *Journal of Experimental Botany*, 2014, 65 (14), pp.3799-3811. <10.1093/jxb/eru039>. <hal-01204073>

**HAL Id: hal-01204073**

**<https://hal.science/hal-01204073v1>**

Submitted on 27 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

## REVIEW PAPER

## Autophagy, plant senescence, and nutrient recycling

Liliana Avila-Ospina<sup>1,2,\*</sup>, Michael Moison<sup>1,2,\*</sup>, Kohki Yoshimoto<sup>1,2</sup> and Céline Masclaux-Daubresse<sup>1,2,†</sup><sup>1</sup> Institut Jean-Pierre Bourgin (JIPB), bat2, UMR 1318, INRA, RD10, 78026 Versailles Cedex<sup>2</sup> AgroParisTech, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France

\* These authors contributed equally to the review.

† To whom correspondence should be addressed. E-mail: [celine.masclaux@versailles.inra.fr](mailto:celine.masclaux@versailles.inra.fr)

Received 24 October 2013; Revised 20 December 2013; Accepted 7 January 2014

## Abstract

Large numbers of publications have appeared over the last few years, dealing with the molecular details of the regulation and process of the autophagy machinery in animals, plants, and unicellular eukaryotic organisms. This strong interest is caused by the fact that the autophagic process is involved in the adaptation of organisms to their environment and to stressful conditions, thereby contributing to cell and organism survival and longevity. In plants, as in other eukaryotes, autophagy is associated with longevity as mutants display early and strong leaf senescence symptoms, however, the exact role of autophagy as a pro-survival or pro-death process is unclear. Recently, evidence that autophagy participates in nitrogen remobilization has been provided, but the duality of the role of autophagy in leaf longevity and/or nutrient recycling through cell component catabolism remains. This review aims to give an overview of leaf senescence-associated processes from the physiological point of view and to discuss relationships between nutrient recycling, proteolysis, and autophagy. The dual role of autophagy as a pro-survival or pro-death process is discussed.

**Key words:** Nitrogen remobilization, plant cell death, proteolysis, Rubisco containing bodies, vesicular trafficking.

## Introduction

In plants, senescence represents the final stage of leaf development. Leaf senescence is characterized by the transition from nutrient assimilation to nutrient remobilization (Masclaux *et al.*, 2000). Catabolic processes occurring during leaf senescence are likely to target chloroplasts since chloroplasts are dismantled in the early phase of senescence while mitochondria remain functional until cell death (Peterson and Huffaker, 1975; Matile, 1992). Depending on plant species, up to 75% of nitrogen is present in the chloroplasts of mesophyll cells (Wittenbach, 1978; Mae *et al.*, 1983; Peoples and Dalling, 1988). Chloroplast dismantling is then a major nitrogen source for nutrient recycling and remobilization. Because nitrogen is essential for plant growth and is one of the most expensive fertilizers used in agriculture, there is a strong interest in studying the mechanisms involved in nitrogen recycling and remobilization in senescing leaves and studies have been undertaken for a long time for that purpose.

However, chloroplast degradation mechanisms are still under debate. Several proteases specifically induced during leaf senescence have been identified (Roberts *et al.*, 2012). Their location in the vacuole then raised the question of substrate trafficking between cell compartments. A vesicular process, the autophagy pathway, has recently been suggested to play a role in chloroplast degradation and nutrient remobilization during natural or stress-induced senescence.

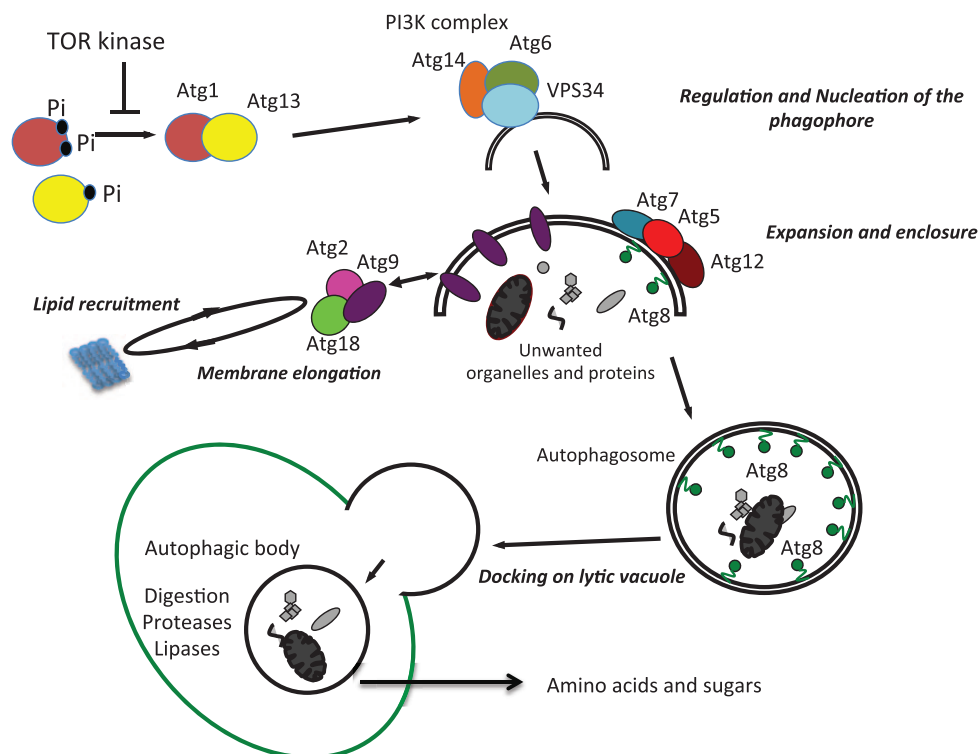
## Autophagy, a vesicular process for nutrient recycling, is present in all eukaryotic cells

The word 'autophagy' is derived from the Greek words; 'auto' meaning 'self' and 'phagein' meaning 'to eat', which was invented by Christian de Duve in 1963. It is an evolutionarily conserved intracellular degradation process whereby

cytoplasmic components are degraded in the vacuole to provide raw materials and energy and also to eliminate damaged or toxic components for the maintenance of essential cellular functions. The existence of autophagy was first suggested by the observation of cytoplasmic material, such as mitochondria and endoplasmic reticulum, being engulfed by double-membrane vesicles and digested by lysosomal enzymes after fusion with lysosomes in the liver of rats starved of amino acids (De Duve and Wattiaux, 1966). In plants, two types of autophagy, called macroautophagy and microautophagy, are well known (Bassham *et al.*, 2006). Macroautophagy, which is most extensively studied, is mediated by a special organelle termed the autophagosome. During macroautophagy, bulk cytosolic constituents and organelles are sequestered into a double membrane structure called an autophagosome. The outer membrane of the autophagosome then fuses with the vacuolar membrane and delivers the inner membrane structure and its cargo, namely the autophagic body, into the vacuolar lumen for degradation. In animals, the fusion of the autophagosome with the lysosome, an acidic compartment containing hydrolytic enzymes, generates the autolysosome. Lysosome-like small vacuole structures have also been found in tobacco suspension-cultured BY-2 cells (Moriyasu and Ohsumi, 1996) but whether they exist in all plant species is still unclear. During microautophagy, a portion of the cytoplasm or some organelle is delivered into the vacuolar lumen by invagination of the vacuolar membrane, causing the formation of intravacuolar vesicles (autophagic bodies), which are then digested by resident vacuolar hydrolases. Usually the

term 'autophagy' indicates macroautophagy unless otherwise specified.

Genetic analyses in the yeast *Saccharomyces cerevisiae* have greatly expanded our knowledge of the molecular mechanisms and physiological roles of autophagy. Notably, the identification of *AuTophagy* (*ATG*) genes, which are essential for autophagosome formation, has contributed significantly to the development of methods that have allowed the molecular dissection of autophagy in higher organisms. The central autophagy machinery consists of 18 *ATG* genes: *ATG1–10*, *12–14*, *16–18*, *29*, and *31* (Fig. 1). In *Arabidopsis* about 30 *ATG* homologues, which correspond to the 18 yeast *ATG* genes, were identified although no homologues have been identified for *ATG14*, *ATG17*, *ATG29*, and *ATG31* (Doelling *et al.*, 2002; Hanaoka *et al.*, 2002; Xiong *et al.*, 2005). In addition to *Arabidopsis*, many *ATG* genes have also been identified in crop and bedding plants such as rice, maize, barley, soybean and petunia (Su *et al.*, 2006; Ghiglione *et al.*, 2008; Chung *et al.*, 2009; Shin *et al.*, 2009; Kuzuoglu-Ozturk *et al.*, 2012; Xia *et al.*, 2012; Shibuya *et al.*, 2013). The function of Atg proteins and their regulation during induction and processes of autophagy have been intensively investigated in yeast, leading to the division of the Atg proteins into four functional groups: (i) the Atg1 protein kinase complex, which is involved in induction and regulation of autophagy; (ii) the PI3 kinase complex specific for autophagy, whose role is to recruit the Atg18–Atg2 complex to autophagic membranes through a Atg18–PI3P interaction; (iii) the Atg9 complex, which is thought to be involved in delivering lipids to



**Fig. 1.** Schematic representation of the function of autophagy proteins (Atg) in yeast. The different complexes involved in autophagosome formation are presented: (i) the TOR (Target of Rapamycin) kinase-dependent phosphorylation of the Atg1/Atg13 regulatory complex inhibits phagophore initiation; (ii) the Atg6/VPS34 PI3K complex recruits the Atg18/Atg2/Atg9 complex for membrane elongation; and (iii) the two Atg12 and Atg8 conjugation complexes participate in the elongation, enclosure, and anchoring of ATG8 into the membrane of autophagosomes.

the pre-autophagosomal structure and the expansion of the initial sequestering structure called phagophore; and (iv) two ubiquitination-like systems, leading to Atg8 lipidation and Atg12 protein conjugation, which are important for the elongation and enclosure steps during autophagosome formation (Fig. 1). Although the *Arabidopsis* ATG proteins (AtATG) do not share high sequence homology with the yeast Atg proteins, most of the essential residues are well conserved, suggesting that the molecular basis of the core autophagic machinery is the same in plants and yeasts. Indeed the ATG8 lipidation system has been shown to be well conserved in *Arabidopsis* and, therefore, ATG8 proteins are useful as molecular markers of autophagosomes in plants as well (Yoshimoto *et al.*, 2004; Contento *et al.*, 2005; Thompson *et al.*, 2005). With the aid of transgenic plants expressing GFP-ATG8, so far, it has been proved that ATG gene-disrupted *Arabidopsis* (*atg* mutants) are defective in autophagy. Although the *atg* mutants can complete their life cycle, they exhibit a strong early leaf senescence phenotype, suggesting that autophagy has important roles in leaf longevity during senescence.

## Leaf senescence is essential for nitrogen use efficiency

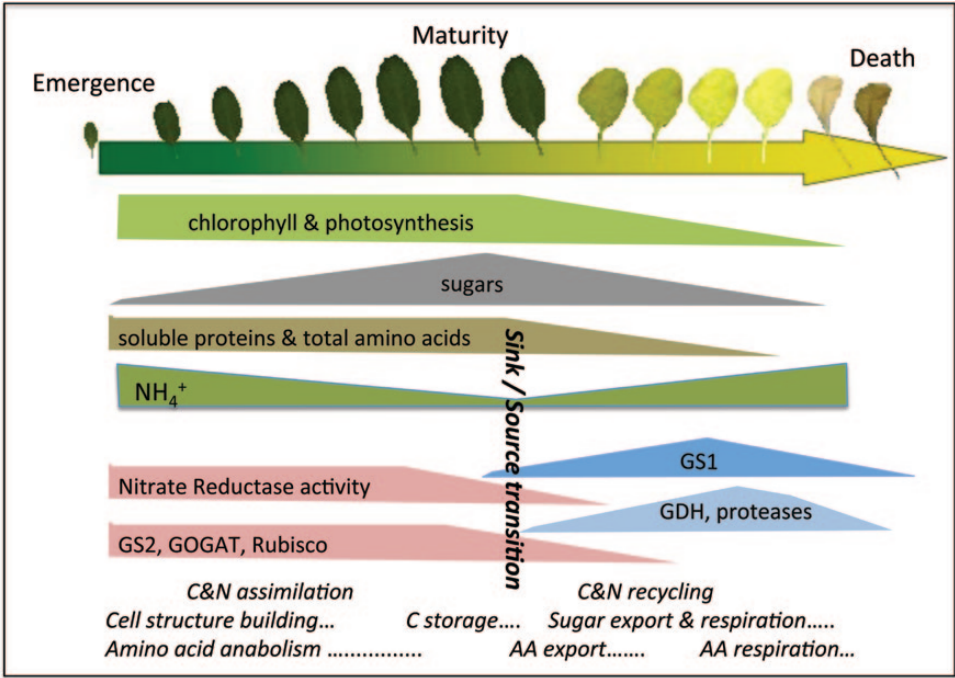
The main macroscopic trait associated with senescence is leaf yellowing. Yellowing of leaves during ageing is symptomatic of major changes that are mainly related to chloroplast decay. The metabolic changes occurring in leaf tissues are associated with an active and regulated degeneration process that seems basically governed by the developmental age and that has certainly been evolutionarily selected (Fischer, 2012; Thomas, 2013). Leaf senescence is an integrated and indispensable part of the plant story that is important for resource management, recycling, and nutrient remobilization efficiency (Guiboileau *et al.*, 2010). Regarding leaf life-span, senescence is also a notable developmental stage as it can last as long as leaf maturation. During senescence, cells do not simply deteriorate and collapse. They undergo orderly changes that modify their structures, their metabolism, and their sink–source relationships (Thomas, 2013). All that the genome-wide analysis performed so far has revealed is that the functions of the senescence-associated genes are notably conserved among plant species and largely involved in the proteolysis and degradation of lipids, cell walls, and nucleic acids, as well as in regulatory processes that can partly be in common with stress responses (Breeze *et al.*, 2011).

As the earliest and most significant change in cell structure is the breakdown of the chloroplast, numerous studies have focused on chloroplast degradation (Sitte, 1977; Mae *et al.*, 1983; Partier, 1988; Chiba *et al.*, 2003). Chloroplast contains up to 70% of the leaf proteins. Thylakoid proteins, light harvesting complexes, photosystems, Calvin cycle proteins, and Rubisco as well as most of the enzymes involved in photorespiration, nitrogen assimilation, and amino acid biosynthesis are localized in the chloroplasts (Peoples and Dalling, 1988). All the studies performed on leaf senescence show that photosynthesis decreases and that, globally, protein and

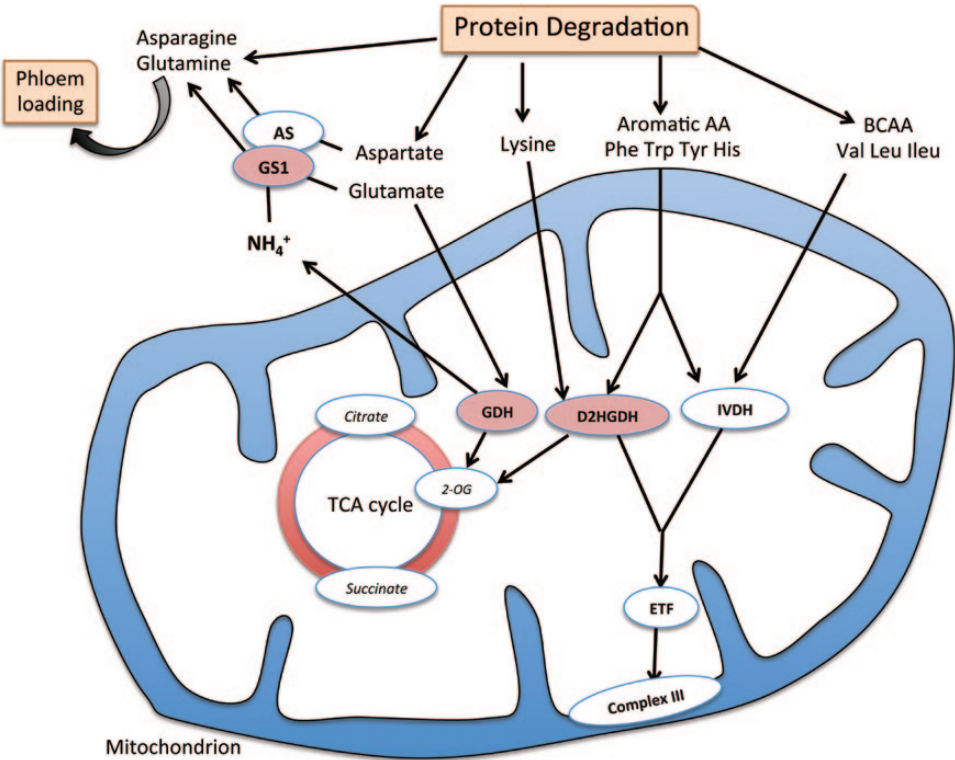
total nitrogen contents also decrease during leaf senescence (Masclaux *et al.*, 2000). Metabolically, it is then likely that carbon and nitrogen assimilation is replaced by the recycling of macromolecules like proteins, RNA, and membrane lipids. It is then assumed that the main function of leaf senescence is to recycle cellular material accumulated during leaf growth and maturation into exportable nutrients to supply developing organs. Leaf senescence, which is understood as a recycling process that contributes to better nutrient management, is then essential for plant productivity (Gregersen *et al.*, 2013).

Leaf senescence occurs in a co-ordinated manner starting from the tip and margins toward the base of the leaf in many plant species like *Arabidopsis*, wheat, barley, and maize. Cellular and molecular events also occur in an orderly manner. From the physiological studies performed on leaf senescence by our group, a picture of the physiological events that occur during the leaf life span can be schematically depicted (Masclaux *et al.*, 2000; Diaz *et al.*, 2008; Fig. 2). On both tobacco and *Arabidopsis*, basically two phases were observed related to the sink–source status of the leaves regarding nitrogen management. During the first period, the leaf expands and, thanks to its active photosynthetic apparatus, becomes autotrophic and efficient in carbon and nitrogen primary assimilation. While sugars are certainly used to build up leaf structures during the first phase of leaf expansion, they start to be stored when the leaf matures. They will certainly be used afterwards for export to new organs and also to support respiration and to sustain energy during senescence when photosynthesis is far less active. Total free amino acid content, which is high during leaf expansion, decreases in parallel to the decrease of chloroplast enzyme activities involved in carbon (Rubisco) and ammonium assimilation (chloroplastic glutamine synthetase GS2). Ammonium, which is the nitrogen donor for all amino acid biosynthesis, is abundant in very young leaves as well as in the oldest ones. The origin of ammonium is certainly different in the two cases, and might be uptake from soil in young leaves and proteolysis and amino acid catabolism in old leaves. Both amino acid catabolism and export certainly occur in parallel in old leaves. The reason for this is that amino acid export efficiency differs depending on the nature of the molecule (Masclaux-Daubresse *et al.*, 2010; Tegeder and Rentsch, 2010). In addition, when leaf cells are depleted of sugars they need to consume amino acids to sustain mitochondria respiration and support cell survival. Ammonium released this way from amino acid catabolism in old leaves then needs to be re-assimilated as glutamine or asparagine, which are two molecules known in many plants as master nitrogen transport forms in the phloem (Fig. 3). Because the chloroplastic GS2 isoform is degraded during senescence as chloroplasts collapse, the cytosolic GS1 isoforms compensate and re-assimilate ammonium for remobilization (Masclaux *et al.*, 2000, 2001). The mitochondrial glutamate dehydrogenase that is known to catabolize glutamate to provide alpha-keto glutarate (2-OG) to the mitochondria is one of the catabolic enzymes releasing ammonium and 2-OG during leaf senescence (Masclaux-Daubresse *et al.*, 2006). While it is now possible to have a better picture of how leaf cells manage nutrient





**Fig. 2.** Schematic representation of the metabolic events occurring in leaves with ageing. Two phases are represented: leaf expansion from emergence to maturity, and leaf senescence from maturity to death. Sink to source transition regarding nitrogen compounds and carbohydrates is characterized by the onset of senescence and by a peak of soluble and starch content in leaves. Chloroplast enzymes, glutamine synthetase/GS Fd-dependent glutamate synthase; GOGAT, (Glutamate OxoGlutarate Amino Transferase), and Rubisco, highly represented in young and photosynthetic tissues, decrease during senescence at the same time as cytosolic glutamine synthetase (GS1), glutamate dehydrogenase (GDH), and proteases are induced. Physiological events (assimilation, anabolism, catabolism, respiration) occurring during leaf expansion and senescence are represented at the bottom of the figure.



**Fig. 3.** Schematic representation of the protein degradation events feeding electrons to the mitochondrial electron transport chain, providing carbon skeletons entering the TCA cycle, and recycling NH<sub>4</sub><sup>+</sup> to support nitrogen remobilization to sink organs. Adapted from Araujo et al. (2011). ETF, electron transfer flavoprotein; IVDH, isovaleryl-CoA dehydrogenase; D2HGDH, 2-hydroxyglutarate dehydrogenase; GDH, glutamate dehydrogenase; GS1, cytosolic glutamine synthetase; AS, asparagine synthetase; BCAA, branched chain amino acids; AA, amino acids; TCA, tricarboxylic acid cycle.

and, especially, nitrogen recycling, the enzymes involved in amino acid catabolism are still under investigation. Recently, Fernies' group proposed that isovaleryl-CoA and 2-hydroxyglutarate, produced through the catabolism of branched chain amino acids, phytol and lysine, respectively, within the chloroplast, are used as substrates by the isovaleryl-CoA and 2-hydroxyglutarate dehydrogenases to act as electron donors for the complex III of the mitochondria respiratory chain (Araujo *et al.*, 2010, 2011). The 2-hydroxyglutarate and the glutamate dehydrogenase genes are all induced during leaf senescence in *Arabidopsis* and these enzymes are good candidates for the replenishment of the TCA cycle with 2-OG during leaf senescence in *Arabidopsis*.

Watanabe *et al.* (2013) published a report dealing with changes in metabolomic profiles in *Arabidopsis* leaf tissues along the basipetal sink–source transition trajectory during senescence. Results presented in this report were consistent with a previous study (Diaz *et al.*, 2005) showing that while nitrate, glutamate, and aspartate amino acids decrease during leaf senescence, stress amino acids (Gaba), branched chain amino acids, and aromatic amino acids accumulate. Carbohydrates such as ceramides, triacylglycerols (TAGs), and polyols are also accumulated during senescence while chloroplast lipids (galactosyldiacylglycerols) are depleted. The decrease of chloroplast lipids has already been used as a marker for leaf senescence as such a decrease is highly correlated with chlorophyll loss (Ferguson and Simon, 1973). Accumulation of TAGs has also been reported previously and is likely to be involved in the mobilization of a carbon source from membrane lipids (Kaup *et al.*, 2002). Interestingly, Watanabe *et al.* (2013) also revealed that the spatiotemporal distribution of tricarboxylic acid cycle (TCA) intermediates was already changed in pre-senescent leaves and that stress-related galactinol and raffinose compounds accumulated in the basal region of the leaves preceding senescence. Changes in TCA intermediates at the onset of leaf senescence certainly reveal changes in the substrates used in the pre-senescent cells for energy. This also suggests that different modalities of the TCA cycle exist and that energy sources might be important for the initiation of the senescence process. The role of respiration in the onset of leaf senescence is supported by the finding that one of the genes controlling senescence onset is *OLD5* that encodes a quinolinate synthase, which is a key enzyme in the *de novo* synthesis of NAD that is essential for cellular redox reactions among which are those of the TCA (Schipper *et al.*, 2008). Another striking result from Watanabe *et al.* (2013) is that an increase of glutamine/glutamate and asparagine/aspartate ratios is observed during leaf senescence, as the absolute concentrations of all these amino acids decrease in senescent leaves. Interconversions of glutamate and aspartate to glutamine and asparagine seem then to be more active during senescence, showing the prominent role of GS1 and AS during senescence (Fig. 3).

The main challenge for leaf tissues during senescence is to manage a progressive degradation of their cellular components without infringement of cell survival, in order to allow nutrient export as long and as deep as possible. Maintenance of mitochondria respiration is essential during the whole

process, from the onset of senescence to cell death. That is surely why the timetable of the degradation of cellular components and of metabolic events is actually tightly controlled.

## Degradation process occurring during leaf senescence targets chloroplast nitrogen resources

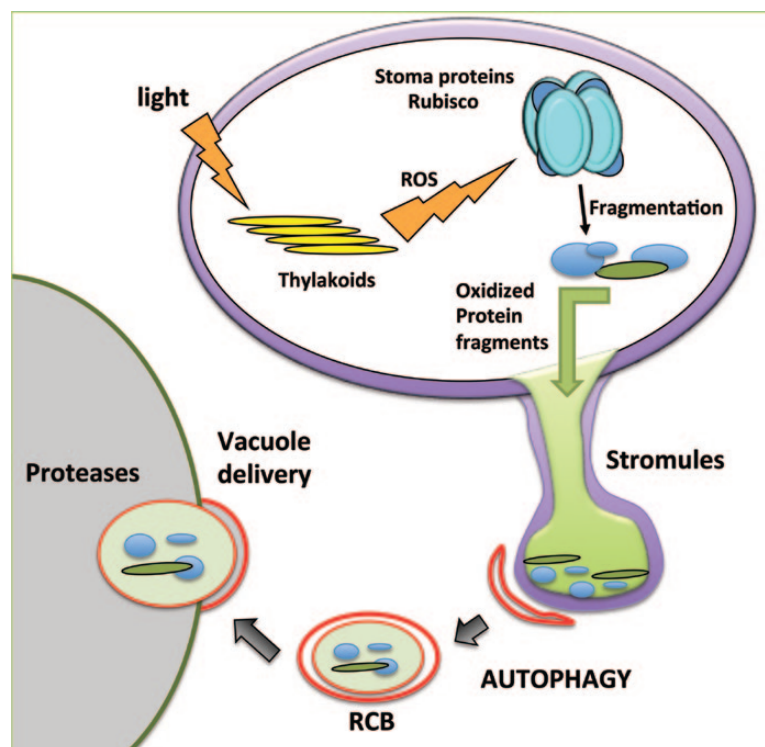
It is well known that protease activities increase during leaf senescence (Feller *et al.*, 1977) and genome-wide studies provided a long list of protease genes that are up-regulated during leaf senescence (Roberts *et al.*, 2012). Among them, the most famous one is the senescence associated gene *SAG12* that encodes a cysteine protease. Many reports describe *SAG12* expression in response to natural leaf senescence and this gene was commonly used as a reference gene for leaf senescence because it was established that the *SAG12* gene is not up-regulated in response to environmental stresses but only in response to ageing. However, little is known about the function of *SAG12* at the cellular level. Guamet's group showed that *SAG12* protein is located in small lytic vacuolar compartments appearing in leaf senescent tissues (Martinez *et al.*, 2008b; Otegui *et al.*, 2005). Such compartments, called senescence-associated vacuoles (SAV), might be related to lysosomal compartments. Although *SAG12* is strongly up-regulated in leaves during senescence, the *sag12* mutant did not show any phenotypes (Otegui *et al.*, 2005). Homologous genes of *SAG12* were also found in other species like tobacco and oilseed rape. In oilseed rape, BnaSAG12 protein is highly abundant in old leaves and especially when nitrogen remobilization is enhanced by a nitrate shortage in the soil (Desclos *et al.*, 2009). This finding suggests that *SAG12* is important for nitrogen remobilization. Many studies have been undertaken with the aim of finding out the proteases involved in chloroplast protein degradation during leaf senescence (see Roberts *et al.*, 2012, for a review). The FtsH protein family whose members are located in the chloroplast and are involved in the degradation of thylakoid proteins might be good candidates. The suggestion that LHCII can be degraded by FtsH6 within the chloroplast was based on data obtained *in vitro*, with thylakoids isolated from wild-type or FtsH6 knock-out plants. However, when LHCII degradation was studied *in vivo* with the same FtsH6 knock-out line (Wagner *et al.*, 2011), it became clear that FtsH6 does not seem to play a crucial role in the degradation of LHCII in senescing leaves, or that there is functional redundancy and other proteases take over LHCII degradation in the FtsH6 knock-out plants. Up to now, none of the *ftsH* mutants isolated so far have presented any abnormality regarding leaf senescence phenotype (Zelisko and Jackowski, 2004; Zelisko *et al.*, 2005). It is therefore likely that the presence of several members of the FtsH family in the chloroplast ensure compensatory effects that could explain such a result.

Investigations on Rubisco and GS2 degradation performed by Feller's and Mae's groups established that attack by Reactive Oxygen Species (ROS) might be a first step in stromal protein degradation. ROS produced by thylakoids under

light exposure were shown to split the Rubisco large subunit and GS2 subunits into fragments whose size was identified in several plant species such as wheat and pea (see Feller *et al.*, 2008, for review). The behaviour of such fragments is not documented; however, García-Ferris and Moreno (1993; 1994) reported that Rubisco proteins or peptides, which have been modified under an oxidative environment, adhere to the membrane of the chloroplasts. One possibility is that chloroplasts expel unwanted material through the budding of small vesicular compartments named Rubisco-containing bodies (RCBs; Chiba *et al.*, 2003). RCBs were also mentioned as Rubisco Vesicular Bodies by some authors (Prins *et al.*, 2008). Such bodies might concentrate detritus like oxidized proteins in order to digest them in more appropriate places like acidic vacuoles. Chiba *et al.* (2003) showed the presence of RCBs in the cytoplasm and vacuole of senescent wheat leaves. The trafficking of stromal proteins from the chloroplast to the vacuole was somewhat consistent with the finding that the aspartic protease CND41 isolated from tobacco is present as an inactive pro-enzyme within the chloroplasts of senescing leaves and can be activated under acidic pH to perform Rubisco degradation (Kato *et al.*, 2004). Therefore, the activation of CND41 in SAVs or the central vacuole could possibly be a way to promote Rubisco degradation. Furthermore, it has long been reported that, in higher plants, most of the acid protease activities in the cells reside in the central vacuole. The trafficking of cellular components from organelles to the lytic vacuoles was then seriously considered as a senescence-related degradation pathway.

As such, the role of autophagy in protein degradation during natural or dark-induced leaf senescence was examined. The first evidence that chloroplast degradation could take place in the vacuoles was provided by electron microscopic observations performed on the protoplasts or cells of senescing leaves (Wittenbach *et al.*, 1982; Chiba *et al.*, 2003). Using plants constitutively expressing stroma-targeted GFP (GFP fused with transit peptide or small subunit of Rubisco), Ishida *et al.* (2008) observed the accumulation of GFP-containing bodies in the vacuolar lumen of leaf cells treated with concanamycin A, a drug that blocks the degradation of autophagic bodies within the central vacuole. As such accumulation cannot be observed in the autophagy mutant *atg5* cells treated with concanamycin A, the authors concluded that RCB trafficking to the vacuole is autophagy-dependent. Using plants co-expressing stroma-targeted RFP and the GFP-ATG8 fusion marker specific for autophagosomes and autophagic bodies, the authors also showed the co-localization of the two fluorescent markers within the vacuole of wild-type leaves (Ishida and Yoshimoto, 2008; Ishida *et al.*, 2008). It is then hypothesized that the first step of stromal protein degradation occurs within the chloroplast and is catalysed by the various uncontrolled ROS released from damaged and inefficient photosynthetic machineries (Fig. 4). Oxidized peptides and proteins released from the chloroplast within RCB structures would then be driven to the vacuole via the autophagy pathway.

Although it seems that autophagy plays a role in stromal protein degradation, other vesicular pathways involving



**Fig. 4.** Hypothetical model of the autophagy-dependent degradation of stroma proteins in senescing leaves. Reactive oxygen species (ROS) produced by photosynthetic antennae lead to the fragmentation and oxidation of stroma proteins like Rubisco. Oxidized fragments that adhere to the membrane accumulate in stromules that bud to form Rubisco-containing bodies (RCB). RCB are taken in charge by autophagosomes that drive them to the central vacuole for degradation.



'senescence-associated vacuoles' (SAVs), different from autophagosomes, might be involved as suggested by [Martinez et al. \(2007, 2008a, b\)](#). SAVs were observed in *Arabidopsis*, soybean, and tobacco. They are acidic and contain intense proteolytic activities, including SAG12. As such, SAVs could be assimilated to autolysosomes. SAVs isolated from tobacco leaves were shown to accumulate stromal proteins such as GS2 and Rubisco but no thylakoid proteins like the proteins D1 and LHCII ([Roberts et al., 2012](#); [Martinez et al., 2008a](#)). The absence of a double membrane and the presence of protease activity and chlorophyll in the lumen of SAVs make them different from autophagosomes. The relationship between SAVs, autophagosomes, and RCB still remains to be clarified, although it seems that all of them participate in stromal protein degradation. The over-accumulation of RCBs in the leaves of transgenic tobacco plants over-expressing the inhibitor of cysteine protease oryzacystatin, shows that cysteine proteases are essential for the degradation of RCBs in the cytosol or in the vacuole ([Prins et al., 2008](#)).

The absence of chlorophyll and thylakoid proteins in RCBs and autophagic bodies also suggests that thylakoid proteins are not degraded through the same pathway as stromal proteins. It is then likely that the protein degradation machinery located within the chloroplast might play a role in thylakoid protein digestion during leaf senescence ([Sakamoto, 2006](#)). In their review, [Roberts et al. \(2012\)](#) report that several chloroplast proteases including Clp, Deg, and FtsH members are up-regulated during natural senescence. However, their roles in the senescence process remain to be explored. Similarly, although few data show that proteasome genes are up-regulated during leaf senescence and that the proteasome can be important in the degradation of oxidized proteins under stress-induced conditions ([Jain et al., 2008](#); [Polge et al., 2009](#); [Roberts et al., 2012](#)), very little is known about its role during leaf senescence. In plants as in animals, the proteasome seems to be mainly involved in the turn-over of short-lived proteins whereas autophagy takes charge of long-lived proteins ([Korolchuk et al., 2010](#)). By contrast with autophagy genes that are globally over-expressed during leaf senescence, the proteasome subunit genes do not seem to be up-regulated during leaf senescence, although a very few exceptions can be found (see [Roberts et al., 2012](#), for a review). It is then more likely that the proteasome participates in the regulation of leaf senescence onset rather than in the mobilization of nutrient resources during leaf senescence ([Raab et al., 2009](#); [Koyama et al., 2013](#)).

## Puzzle of autophagy role in cell death and longevity

As reported in the previous sections, autophagy was first described in the 1960s by de Duve who observed that, under amino acid starvation, rat liver cells accumulated autophagosomes containing a large variety of cell components (protein aggregates, mitochondria, endoplasmic reticulum fragments). Autophagy was thought to be a mechanism involved in cell auto-digestion and then in the cell death process. Further

reports proposed that the autophagy cell death-promoting process can balance the apoptosis process when needed ([Maiuri et al., 2007](#)). Although high autophagic activity can contribute to cell death under certain unusual conditions ([Kabbage et al., 2013](#)), the main function of autophagy in all organisms is to cope with unwanted cell components and to maintain cell homeostasis and longevity. Under stressful conditions, as well as during leaf senescence, autophagic activity is increased in order to clean up the cell from oxidized proteins, damaged organelles, and other toxic compounds ([Fig. 5](#); [Xiong et al., 2007a, b](#); [Breeze et al., 2011](#)). While, for a long time, it was considered that autophagy is a bulk degradation process that removes and recycles cell components unselectively, there is now much evidence to suggest that autophagy is selective and targets different cell components depending on environmental signals, stress intensity, and cell adaptation needs ([Svenning et al., 2011](#); [Zientara-Rytter et al., 2011](#)).

The enigma of the role of autophagy in leaf senescence is due to the remaining idea in literature that autophagy is associated with cell death. It was considered in several reports that the increase of autophagic activity during leaf ageing promotes leaf senescence and cell death through the degradation of chloroplasts and other cellular structures. Even though autophagy is certainly contributing to the large-scale cell component degradation process that will lead to cell death at the end, it is probable that, during the first steps of the leaf senescence programme, autophagy works to maintain cell homeostasis, keep cells healthy, taking in charge cell component turn-over, and avoiding cell damage due to oxidative stress. Supporting this hypothesis there is evidence that organisms with a low autophagic capacity, i.e. *atg* mutants, are more sensitive to stresses and show precocious leaf senescence phenotypes and earlier cell death ([Phillips et al., 2008](#)).

Depending on its induction during leaf development, autophagy might be a triggering factor of senescence onset or a process induced by leaf senescence for nutrient remobilization. The fine-tuning of the balance of autophagic activity and cell damage level might indeed be important to control the onset of leaf senescence. On the other hand, evidence was recently provided that autophagy plays a role in nitrogen remobilization to the seeds in *Arabidopsis*. Although transcript levels of *ATG* genes are not completely appropriate to monitor autophagy level because there are several levels of post-translational regulation, the expression was examined of several *ATG* genes, of N remobilization markers, and of the *SAG12* senescence marker in parallel in the six first leaves of two recombinant inbred lines of *Arabidopsis* previously characterized by our group with regard to N remobilization and leaf senescence ([Diaz et al., 2005, 2008](#)). These RIL083 and RIL272 lines have been shown to display early and late leaf senescence phenotypes, respectively, but are similar in flowering time ([Fig. 6](#)), rosette size, and reproductive organ development. On the six first leaves of these lines, *SAG12*, *ATGs*, and *GSs* transcripts were quantified ([Fig. 6](#)). The relative expression of each gene was normalized according to its lowest expression level in order to facilitate representation of the results. As expected, the transcript level of *SAG12* was increased



A	natural	dark induced	detached leaf
ATG3	1.75	2.27	2.52
ATG4b	1.71	1.87	2.69
ATG5	2.65	2.16	3.45
ATG6	1.93	2.28	2.52
ATG7	3.92	16.95	9.90
ATG8a	2.61	7.99	9.87
ATG8b	1.63	4.38	3.13
ATG8c	1.71	2.09	2.67
ATG8d	1.25	1.96	2.66
ATG8e	6.39	7.72	5.67
ATG8f	1.19	1.35	1.77
ATG8g	1.24	2.42	2.50
ATG8h	2.80	12.04	7.40
ATG8i	1.87	2.23	3.22
ATG9	2.75	6.80	5.26
ATG12b	2.36	1.80	3.37
ATG18a	2.02	3.25	3.79
ATG18c	1.16	1.44	1.28
ATG18d	1.35	1.13	1.31
ATG18g	1.08	1.28	1.10
ATG18h	2.99	4.89	4.91

B	F test Probability
ATG1c	0.000
ATG13a	0.000
ATG13b	0.000
ATG2	0.000
ATG3	0.000
ATG4b	0.014
ATG6	0.000
ATG7	0.000
ATG9	0.000
ATG8f	0.000
ATG8g	0.000
ATG8h	0.000
ATG8i	0.000
ATG18a	0.001
ATG18c	0.000
ATG18f	0.000

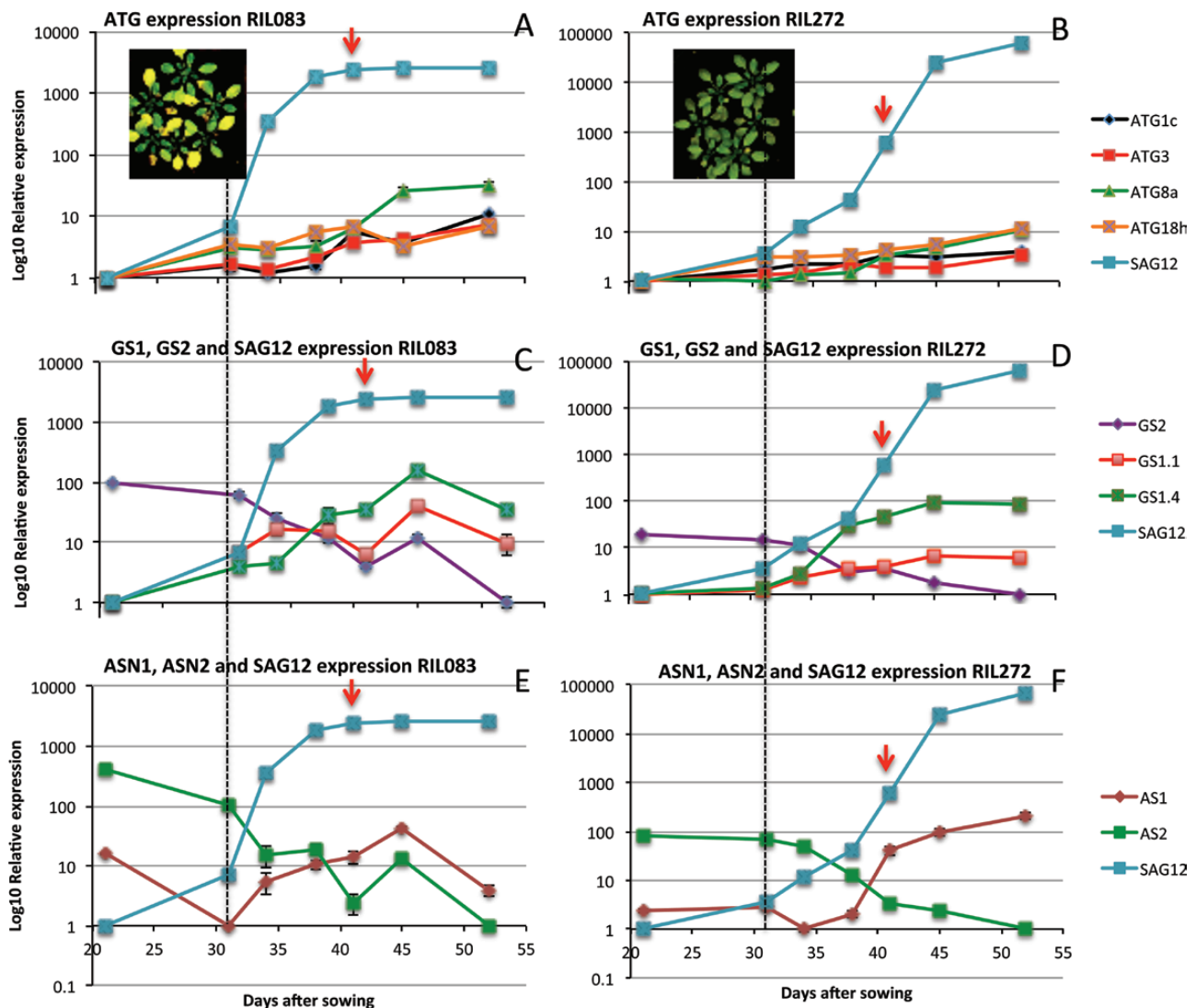
**Fig. 5.** Expression of autophagy genes in *Arabidopsis* during natural and stress-induced senescence. Fold change in the expression of autophagy genes in *Arabidopsis* during natural senescence (estimated as 75% of chlorophyll decrease), 9 d of dark-induced senescence and 6 d of detached leaf-induced senescence (data from [van der Graaff et al., 2006](#)). ATG genes identified as differentially expressed during leaf senescence are shown, together with their statistical significance for differential expression based on the *F* test analysis (*P* value following MTC), (data from [Breeze et al., 2011](#))

later in the stay-green RIL272 than in the early-senescing RIL083. Regarding autophagy genes, it was observed that, in both genotypes, transcript levels of *ATGs* were increased later and more steadily than that of *SAG12*. The *ATGs* transcript level was also globally lower in RIL272 than RIL083. Interestingly, in RIL083, the increase of hte *ATG8a* mRNA level was higher after flowering time. By contrast, transcripts of nitrogen remobilization-related genes (*GS1.1*, *GS1.4*, and *ASN1*) were increased simultaneously and in parallel with *SAG12*. Also, the profiles of the senescence-repressed genes, *GS2* and *ASN2*, appeared to be well co-ordinated with the *SAG12* expression profile. From these profiles, it is possible to have an idea of autophagy induction during leaf development and it seems that, at the transcriptional level at least, autophagy is increased during late senescence, after *SAG12* and also after the N remobilization markers. Autophagy also seems more induced in the earlier-senescing line than in the later-senescing one. In the senescing RIL083, an increase of the *ATG8a* mRNA steady-state level after flowering is consistent with the role of autophagy in N remobilization to the seeds. All these results argue in favour of the induction of autophagy by leaf senescence and not the reverse. Data of the report by [Guiboileau et al. \(2012\)](#) are also in agreement with the role of autophagy in nutrient recycling and remobilization from sinks to sources. Since autophagy genes are induced after *SAG12*, it seems unlikely that autophagy increase anticipates leaf senescence. The establishment of methods to monitor autophagic activity should help to emphasize these results.

Autophagy plays a role in nutrient remobilization during leaf senescence

The reason for our interest in the role of autophagy in nutrient mobilization during senescence is explained by the accumulation of evidence that autophagy might manage the turn-over of long-life proteins and nutrient recycling for remobilization. The function of autophagy that was described in animals and yeast, i.e. a bulk degradative process involved in the degradation of long-life proteins, is consistent with this idea and with the degradation of Rubisco, a long-life protein in plants. It is also consistent with the degradation of damaged organelles as described by De Duve in the early 1960s. Induction of autophagy during senescence, under nitrogen shortage and in response to drought or salt stress, is concomitant with the occurrence of nutrient remobilization in leaves. Although several reports have assumed that autophagy is involved in nutrient recycling in plants; however, there were no physiological data to support this hypothesis until nitrogen remobilization and C/N metabolism in autophagy mutants was recently investigated ([Guiboileau et al., 2012, 2013](#)).

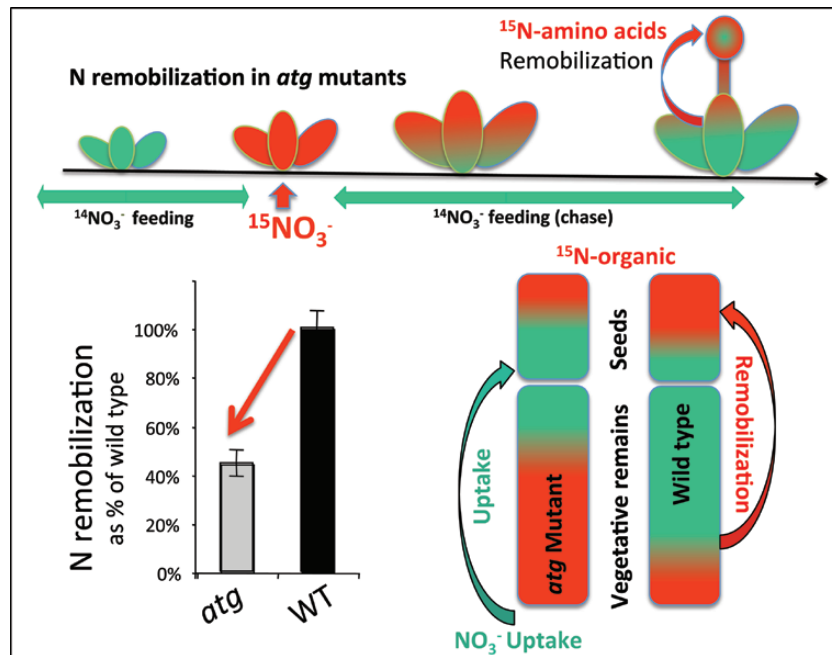
Using <sup>15</sup>N tracing in pulse/chase experiments previously improved in *Arabidopsis* by our group ([Lemaitre et al., 2008; Diaz et al., 2008; Masclaux-Daubresse and Chardon, 2011](#)), it was shown that nitrogen remobilization efficiency is largely reduced in autophagy mutants (*atg*) compared with the wild type ([Fig. 7; Guiboileau et al., 2012](#)). The defect of N remobilization in *atg* mutants was observed independently of the nitrate availability in the soil and it can partially explain the



**Fig. 6.** Comparison of the expression profiles of autophagy and nitrogen remobilization genes with the *SAG12* senescence marker. Transcripts coding for autophagy ATG1c, ATG3, ATG8a, and ATG18h (A, B), glutamine synthetase GS2, GS1.1, and GS1.4 (C, D), and asparagine synthetase AS1 and AS2 (E, F) proteins were quantified using Q-PCR in the six first leaves of the early senescing RIL083 line (A, C, E) and in the six first leaves of the late senescing RIL272 line (B, D, F). Mean and standard deviation are shown ( $n=3$ ).

lower biomass and yield production observed in *atg* mutants. Indeed the altered biomass, yield, and N remobilization in *atg* might be due to the shorter life span and the severe and rapid leaf senescence of the mutants. As leaf senescence in *atg* mutants is due to an excess of salicylic acid production (Yoshimoto *et al.*, 2009), N remobilization efficiency was monitored in the double mutant *atg5 sid2* and in the NahG *atg5* transformant. Similar defects for N remobilization and yield were found in both plants without detecting any differences in leaf senescence relative to the wild type. From the data collected, it was evaluated that autophagy is involved in 40% of the  $^{15}\text{N}$  remobilization flux from the rosette to the seeds of plants grown under low-nitrate conditions. A defect of ATG protein-dependent autophagic activity does not block the whole nitrogen recycling/remobilization flux. Other remobilization pathways remain active in *atg* mutants. This shows that autophagy does not take charge of all the substrates recycled for remobilization, and that autophagy degradation is certainly selective for some nitrogen sources. The study of nitrogen pools

present in the leaves of *atg* mutants during ageing shows that mutants actually accumulate all sorts of nitrogen compounds (amino acids, proteins, and ammonium) except nitrate. The absence of nitrate accumulation in *atg*, together with the higher N/C ratio measured in *atg* mutants, indicates that *atg* mutants are not affected in nitrate uptake or in the nitrate reduction pathway. By contrast, the higher concentrations of amino acid, soluble protein, and ammonium measured in leaves of *atg* mutants show that the recycling or the export of organic nitrogen compounds is affected. In order to determine whether impaired proteolysis can explain protein over-accumulation in *atg* mutants, protease activities were measured (Guiboileau *et al.*, 2013). Surprisingly, it was found that the activities of carboxypeptidase, aminopeptidase, and endopeptidase were increased in the *atg* mutants relative to the wild type in parallel with the accumulation of soluble proteins. However, it was found that all proteins did not accumulate in a similar manner in *atg* leaves compared with the wild type. Ribosomal proteins L13 and S6 were specifically over-accumulated in the *atg*



**Fig. 7.** Nitrogen remobilization defects in autophagy mutants. Using  $^{15}\text{N}$  tracing according to [Lemaitre \*et al.\* \(2008\)](#), the N remobilization efficiency (partitioning of  $^{15}\text{N}$  in seeds normalized according to harvest index value) was estimated in *atg* mutants and compared with the wild type ([Guiboileau \*et al.\*, 2012](#)).

mutants. In addition, peptides as cleavage products released from a deep degradation of Rubisco and GS2 proteins were more abundant in *atg* mutants, suggesting an involvement of autophagy in the degradation of stromal chloroplast proteins. All these accumulations of specific proteins emphasize the idea that autophagy is specific to some proteins ([Guiboileau \*et al.\*, 2013](#); [Lee \*et al.\*, 2013](#)).

### Autophagy and petal senescence: a cell death programme of nutrient remobilization?

Petal senescence has been studied for a long time because it shortens the vase life of cut flowers. Studies focusing on nutrient resources able to sustain mitochondrial respiration in petals after pollination or after cutting have been documented in many plant species ([Kaltaler and Steponkus, 1976](#); [Skubatz \*et al.\*, 1989](#); [Wulster \*et al.\*, 1984](#)). In addition to the degradation of cellular components to provide sugars, amino acids, and lipid molecules for respiration, it was shown that both macro and microelements are exported from the senescing corolla through the phloem saps ([Verlinden, 2003](#); [Chapin and Jones, 2009](#)). In addition, it is possible that the remobilization of some macro and micro elements was more efficient during pollination induced-senescence than during petal senescence of unpollinated flowers (see [Jones, 2013](#), for a review). Such a difference showed that petal senescence is triggered by pollination and by the development of the ovary as a new sink that drives nutrients ([Hew \*et al.\*, 1989](#)).

Involvement of autophagy in petal senescence has been proposed for a very long time since numerous vesicles and

cytoplasmic components were observed in the vacuoles of senescing petals by electron microscopy ([Matile and Winkensbach, 1971](#)). Recently, a possible role of autophagy in petal senescence was documented at the molecular level. In the petals of morning glory and petunia, transcript levels of *ATG* genes such as *ATG4* and *ATG8* were increased during senescence ([Yamada \*et al.\*, 2009](#); [Shibuya \*et al.\*, 2011, 2013](#)). Interestingly, such *ATG* genes were up-regulated by ethylene treatment that is well known as an inducer of petal senescence. Since this induction was very quick, after just 4 h of ethylene treatment, the expression of the genes seemed to be regulated more directly by ethylene during petal senescence. However, whether the induction of the transcript levels of *ATG* genes do correlate with autophagic activity remains to be confirmed. Monodansylcadaverine (MDC)-stained vesicles were shown to appear during petal senescence in both morning glory and petunia, suggesting that autophagy is induced during petal senescence since MDC had been thought to stain autophagic structures ([Shibuya \*et al.\*, 2011, 2013](#)). However, recent studies in mammalian cells revealed that MDC is not a suitable marker for autophagy-specific structures. MDC-positive structures did not always co-localize with autophagosomes labelled by GFP-ATG8, which is the most reliable molecular marker for autophagosomes ([Mizushima \*et al.\*, 2002](#)). It seems that MDC labels acidic organelles such as lysosomes and late endosomes rather than autophagosomes. Therefore, careful analysis of petal senescence-induced MDC-stained structures will be required. It could be that autophagy induced during petal senescence may be ATG protein independent. Current data using detached flower assays support the hypothesis that autophagy is induced during petal senescence to remobilize nutrients from petals to developing ovaries ([Shibuya \*et al.\*, 2013](#)), but further



confirmation is required using *ATG* gene knock-out mutants or virus-induced *ATG* gene-silencing plants. In addition, it is still unclear whether autophagy is a direct cause of cell death in petal senescence. In *Ipomoea* petals, 3-methyladenine (3-MA) accelerated the time to visual senescence symptoms (Yamada *et al.*, 2009). 3-MA is a potent inhibitor of phosphoinositide 3-kinase (PI3K), resulting in the inhibition of the formation of autophagic structures. This result suggests that autophagy during *Ipomoea* petal senescence is not the cause of cell death, but rather is a factor of cell survival in the same way as in leaf senescence. However, it cannot be excluded that 3-MA inhibits not only autophagy but also other pathways since PI3K has pleiotropic functions. Autophagy-defective bedding plants generated by knocking out or knocking down *ATG* genes will provide a more precise answer to whether autophagy has a pro-survival function or a pro-death function in petal senescence.

## Conclusion

There is much evidence to show that autophagy plays a role in nutrient recycling and nutrient management at the whole plant level. Evident relationships exist between autophagy and leaf senescence. However, the co-ordination of the different processes involved in protein degradation, chloroplast dismantling, and nutrient mobilization, and the cause and effect relationships are not always clear. Studies interested in the finding of autophagy targets and in the engineering of plants that can display different autophagic capacities would facilitate advances in the comprehension of the role of autophagy during leaf, petal, and even root senescence.

## Acknowledgements

This work was supported by the FP7 Marie Curie Actions—Networks for Initial Training Project FP7-MC-ITN No. 264394 (acronym CropLife) for LA-O, by CETIOM (Centre Technique des Oléagineux) and Biologie et Amélioration des Plantes, INRA, for MM, and by an INRA Package programme for KY. The authors would like to thank Ekatarina Merculova for proof reading the English.

## References

- Araujo WL, Ishizaki K, Nunes-Nesi A, *et al.* 2010. Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of Arabidopsis mitochondria. *The Plant Cell* **22**, 1549–1563.
- Araujo WL, Tohge T, Ishizaki K, Leaver CJ, Fernie AR. 2011. Protein degradation: an alternative respiratory substrate for stressed plants. *Trends in Plant Science* **16**, 489–498.
- Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K. 2006. Autophagy in development and stress responses of plants. *Autophagy* **2**, 2–11.
- Breeze E, Harrison E, McHattie S, *et al.* 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. *Plant Cell* **23**, 873–894.
- Chapin LJ, Jones ML. 2009. Ethylene regulates phosphorus remobilization and expression of a phosphate transporter (PhPT1) during petunia corolla senescence. *Journal of Experimental Botany* **60**, 2179–2190.
- Chiba A, Ishida H, Nishizawa NK, Makino A, Mae T. 2003. Exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. *Plant and Cell Physiology* **44**, 914–921.
- Chung T, Suttangkakul A, Vierstra RD. 2009. The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8–lipid adduct are regulated by development and nutrient availability. *Plant Physiology* **149**, 220–234.
- Contento AL, Xiong Y, Bassham DC. 2005. Visualization of autophagy in Arabidopsis using the fluorescent dye monodansylcadaverine and a GFP-AtATG8e fusion protein. *The Plant Journal* **42**, 598–608.
- De Duve C, Wattiaux R. 1966. Functions of lysosomes. *Annual Review of Physiology* **28**, 435–492.
- Desclos M, Etienne P, Coquet L, Jouenne T, Bonnefoy J, Segura R, Reze S, Ourry A, Avice JC. 2009. A combined <sup>15</sup>N tracing/proteomics study in *Brassica napus* reveals the chronology of proteomics events associated with N remobilisation during leaf senescence induced by nitrate limitation or starvation. *Proteomics* **9**, 3580–3608.
- Diaz C, Lemaitre T, Christ A, Azzopardi M, Kato Y, Sato F, Morot-Gaudry JF, Le Dily F, Masclaux-Daubresse C. 2008. Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in Arabidopsis under low nitrogen nutrition. *Plant Physiology* **147**, 1437–1449.
- Diaz C, Purdy S, Christ A, Morot-Gaudry JF, Wingler A, Masclaux-Daubresse CL. 2005. Characterization of markers to determine the extent and variability of leaf senescence in Arabidopsis. A metabolic profiling approach. *Plant Physiology* **138**, 898–908.
- Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD. 2002. The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. *Journal of Biological Chemistry* **277**, 33105–33114.
- Feller U, Song T, Hageman R. 1977. Leaf proteolytic activities and senescence during grain development of field-grown corn (*Zea mays* L.). *Plant Physiology* **59**, 290–294.
- Feller U, Anders I, Mae T. 2008. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany* **59**, 1615–1624.
- Ferguson C, Simon E. 1973. membrane lipids in senescing green tissue. *Journal of Experimental Botany* **24**, 307–316.
- Fischer AM. 2012. The complex regulation of senescence. *Critical Reviews in Plant Sciences* **31**, 124–147.
- García-Ferris C, Moreno J. 1993. Redox regulation of enzymatic activity and proteolysis and susceptibility of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Euglena gracilis*. *Photosynthesis Research* **35**, 55–66.
- García-Ferris C, Moreno J. 1994. Oxidative modification and breakdown of ribulose-1,5-bisphosphate carboxylase/oxygenase induced in *Euglena gracilis* by nitrogen starvation. *Planta* **193**, 208–215.
- Ghiglione HO, Gonzalez FG, Serrago R, Maldonado SB, Chilcott C, Cura JA, Miralles DJ, Zhu T, Casal JJ. 2008. Autophagy regulated by day length determines the number of fertile florets in wheat. *The Plant Journal* **55**, 1010–1024.
- Gregersen P, Culetic A, Boschian L, Krupinska K. 2013. Plant senescence and crop productivity. *Plant Molecular Biology* **82**, 603–622.
- Guiboileau A, Avila-Ospina L, Yoshimoto K, Soulay F, Azzopardi M, Marmagne A, Lothier J, Masclaux-Daubresse C. 2013. Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in Arabidopsis leaves depending on nitrate availability. *New Phytologist* **199**, 683–694.
- Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C. 2010. Senescence and death of plant organs: nutrient recycling and developmental regulation. *Comptes Rendus Biologies* **333**, 382–391.
- Guiboileau A, Yoshimoto K, Soulay F, Bataille M-P, Avice J-C, Masclaux-Daubresse C. 2012. Autophagy machinery controls nitrogen remobilization at the whole-plant level under both limiting and ample nitrate conditions in Arabidopsis. *New Phytologist* **194**, 732–740.
- Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, Shibata D, Tabata S, Ohsumi Y. 2002. Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an Arabidopsis autophagy gene. *Plant Physiology* **129**, 1181–1193.



- Hew CS, Tan SC, Chin TY, Ong TK.** 1989. Influence of ethylene on enzyme activities and mobilization of materials in pollinated *Arachnis* orchid flowers. *Journal of Plant Growth Regulation* **8**, 121–130.
- Ishida H, Yoshimoto K.** 2008. Chloroplasts are partially mobilized to the vacuole by autophagy. *Autophagy* **4**, 961–962.
- Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, Makino A, Ohsumi Y, Hanson MR, Mae T.** 2008. Mobilization of rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. *Plant Physiology* **148**, 142–155.
- Jain V, Kaiser W, Huber SC.** 2008. Cytokinin inhibits the proteasome-mediated degradation of carbonylated proteins in *Arabidopsis* leaves. *Plant and Cell Physiology* **49**, 843–852.
- Jones M.** 2013. Mineral nutrient remobilization during corolla senescence in ethylene-sensitive and -insensitive flowers. *AoB Plants* DOI: 010.1093/aobpla/plt023
- Kabbage M, Williams B, Dickman MB.** 2013. Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of sclerotinia sclerotiorum. *PLoS Pathogens* **9**, e1003287. doi:1003210.1001371/journal.ppat.1003287.
- Kaltaler REL, Steponkus PL.** 1976. Factors affecting respiration in cut roses. *Journal of the American Society for Horticultural Science* **101**, 353–354.
- Kato Y, Murakami S, Yamamoto Y, Chatani H, Kondo Y, Nakano T, Yokota A, Sato F.** 2004. The DNA-binding protease, CND41, and the degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase in senescent leaves of tobacco. *Planta* **220**, 97–104.
- Kaup MT, Froese CD, Thompson JE.** 2002. A role for diacylglycerol acyltransferase during leaf senescence. *Plant Physiology* **129**, 1616–1626.
- Korolchuk VI, Menzies FM, Rubinsztein DC.** 2010. Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. *FEBS Letters* **584**, 1393–1398.
- Koyama T, Nii H, Mitsuda N, Ohta M, Kitajima S, Ohme-Takagi M, Sato F.** 2013. A regulatory cascade involving class II ETHYLENE RESPONSE factor transcriptional repressors operates in the progression of leaf senescence. *Plant Physiology* **162**, 991–1005.
- Kuzuoglu-Ozturk D, Yalcinkaya OC, Akpinar BA, Mitou G, Korkmaz G, Gozuacik D, Budak H.** 2012. Autophagy-related gene, *TdAtg8*, in wild emmer wheat plays a role in drought and osmotic stress response. *Planta* **236**, 1081–1092.
- Lee TA, Vande Wetering SW, Brusslan JA.** 2013. Stromal protein degradation is incomplete in *Arabidopsis thaliana* autophagy mutants undergoing natural senescence. *BMC Research Notes* **6**, 17–17.
- Lemaitre T, Gaufichon L, Boutet-Mercey S, Christ A, Masclaux-Daubresse C.** 2008. Enzymatic and metabolic diagnostic of nitrogen deficiency in *Arabidopsis thaliana* Wassilewskija accession. *Plant and Cell Physiology* **49**, 1056–1065.
- Mae T, Makino A, Ohira K.** 1983. Changes in the amounts of ribulose biphosphate carboxylase synthesized and degraded during the life span of rice leaf (*Oryza sativa* L.). *Plant and Cell Physiology* **24**, 1079–1086.
- Maiuri MC, Zalckvar E, Kimchi A, Kroemer G.** 2007. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nature Reviews Molecular Cell Biology* **8**, 741–752.
- Martinez DE, Bartoli CG, Grbic V, Guamet JJ.** 2007. Vacuolar cysteine proteases of wheat (*Triticum aestivum* L.) are common to leaf senescence induced by different factors. *Journal of Experimental Botany* **58**, 1099–1107.
- Martinez DE, Costa ML, Gomez FM, Otegui MS, Guamet JJ.** 2008a. 'Senescence-associated vacuoles' are involved in the degradation of chloroplast proteins in tobacco leaves. *The Plant Journal* **56**, 196–206.
- Martinez DE, Costa ML, Guamet JJ.** 2008b. Senescence-associated degradation of chloroplast proteins inside and outside the organelle. *Plant Biology (Stuttgart)* **10**, Supplement 1, 15–22.
- Masclaux C, Quillere I, Gallais A, Hirel B.** 2001. The challenge of remobilization in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Annals of Applied Biology* **138**, 69–81.
- Masclaux C, Valadier MH, Brugiere N, Morot-Gaudry JF, Hirel B.** 2000. Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. *Planta* **211**, 510–518.
- Masclaux-Daubresse C, Chardon F.** 2011. Exploring nitrogen remobilization for seed filling using natural variation in *Arabidopsis thaliana*. *Journal of Experimental Botany* **62**, 2131–2142.
- Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A.** 2010. Nitrogen uptake, assimilation, and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany* **105**, 1141–1157.
- Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A.** 2006. Glutamine synthetase–glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink–source nitrogen cycle in tobacco. *Plant Physiology* **140**, 444–456.
- Matile P.** 1992. Chloroplast senescence. In: Baker NR, Thomas H, eds. *Crop photosynthesis: spatial and temporal determinants*. Amsterdam: Elsevier, 413–440.
- Matile P, Winkenbach F.** 1971. Function of lysosomes and lysosomal enzymes in the senescing corolla of the morning glory (*Ipomoea purpurea*). *Journal of Experimental Botany* **22**, 759–771.
- Mizushima N, Yamamoto A, Yoshimori T, Ohsumi Y.** 2002. Mice with a fluorescent marker for autophagy. *Molecular Biology of the Cell* **13**, 750.
- Moriyasu Y, Ohsumi Y.** 1996. Autophagy in tobacco suspension-cultured cells in response to sucrose starvation. *Plant Physiology* **111**, 1233–1241.
- Otegui MS, Noh YS, Martinez DE, Vila Petroff MG, Andrew Staehelin L, Amasino RM, Guamet JJ.** 2005. Senescence-associated vacuoles with intense proteolytic activity develop in leaves of *Arabidopsis* and soybean. *The Plant Journal* **41**, 831–844.
- Partier B.** 1988. Gerontoplasts: the yellow end in the ontogenesis of chloroplasts. *Endocytobiosis and Cell Research* **5**, 163–190.
- Peoples M, Dalling M.** 1988. The interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. In: Noodén L, Leopold A, eds. *Senescence and aging in plants*. San Diego: Academic Press, 181–217.
- Peterson LW, Huffaker RC.** 1975. Loss of ribulose 1,5-diphosphate carboxylase in proteolytic activity during senescence of detached primary barley leaves. *Plant Physiology* **55**, 1009–1015.
- Phillips AR, Suttangkakul A, Vierstra RD.** 2008. The ATG12-conjugating enzyme ATG10 is essential for autophagic vesicle formation in *Arabidopsis thaliana*. *Genetics* **178**, 1339–1353.
- Polge C, Jaquinod M, Holzer F, Bourguignon J, Walling L, Brouquisse R.** 2009. Evidence for the existence in *Arabidopsis thaliana* of the proteasome proteolytic pathway ACTIVATION IN RESPONSE TO CADMIUM. *Journal of Biological Chemistry* **284**, 35412–35424.
- Prins A, van Heerden PDR, Olmos E, Kunert KJ, Foyer CH.** 2008. Cysteine proteinases regulate chloroplast protein content and composition in tobacco leaves: a model for dynamic interactions with ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) vesicular bodies. *Journal of Experimental Botany* **59**, 1935–1950.
- Raab S, Drechsel G, Zarepour M, Hartung W, Koshiba T, Bittner F, Hoth S.** 2009. Identification of a novel E3 ubiquitin ligase that is required for suppression of premature senescence in *Arabidopsis*. *The Plant Journal* **59**, 39–51.
- Roberts IN, Caputo C, Criado MV, Funk C.** 2012. Senescence-associated proteases in plants. *Physiologia Plantarum* **145**, 130–139.
- Sakamoto W.** 2006. Protein degradation machineries in plastids. *Annual Review of Plant Biology* **57**, 599–621.
- Schippers JHM, Nunes-Nesi A, Apetrei R, Hille J, Fernie AR, Dijkwel PP.** 2008. The *Arabidopsis* onset of leaf death5 mutation of quinolinate synthase affects nicotinamide adenine dinucleotide biosynthesis and causes early ageing. *The Plant Cell* **20**, 2909–2925.
- Svenning S, Lamark T, Krause K, Johansen T.** 2011. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. *Autophagy* **7**, 993–1010.
- Shibuya K, Niki T, Ichimura K.** 2013. Pollination induces autophagy in petunia petals via ethylene. *Journal of Experimental Botany* **64**, 1111–1120.
- Shibuya K, Shimizu K, Yamada T, Ichimura K.** 2011. Expression of autophagy-associated ATG8 genes during petal senescence in Japanese morning glory. *Journal of the Japanese Society for Horticultural Science* **80**, 89–95.

- Shin J-H, Yoshimoto K, Ohsumi Y, Jeon J-s, An G.** 2009. OsATG10b, an autophagosome component, is needed for cell survival against oxidative stresses in rice. *Molecules and Cells* **27**, 67–74.
- Sitte P.** 1977. Chromoplasten—bunte Objekte der modernen Zellbiologie. *Biologie in unserer Zeit* **7**, 65–74.
- Skubatz H, Meeuse BJD, Bendich AJ.** 1989. Oxidation of proline and glutamate by mitochondria of the inflorescence of voodoo lily (*Sauromatum guttatum*). *Plant Physiology* **91**, 530–535.
- Su W, Ma H, Liu C, Wu J, Yang J.** 2006. Identification and characterization of two rice autophagy associated genes, *OsAtg8* and *OsAtg4*. *Molecular Biology Reports* **33**, 273–278.
- Tegeder M, Rentsch D.** 2010. Uptake and partitioning of amino acids and peptides. *Molecular Plant* **3**, 997–1011.
- Thomas H.** 2013. Senescence, ageing and death of the whole plant. *New Phytologist* **197**, 696–711.
- Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD.** 2005. Autophagic nutrient recycling in Arabidopsis directed by the ATG8 and ATG12 conjugation pathways. *Plant Physiology* **138**, 2097–2110.
- van der Graaff E, Schwacke R, Schneider A, Desimone M, Flugge UI, Kunze R.** 2006. Transcription analysis of Arabidopsis membrane transporters and hormone pathways during developmental and induced leaf senescence. *Plant Physiology* **141**, 776–792.
- Verlinden S.** 2003. Changes in mineral nutrient concentrations in petunia corollas during development and senescence. *HortScience* **38**, 71–74.
- Wagner R, Aigner H, Pružinská A, Jänkänpää H, Jansson S, Funk C.** 2011. Fitness analyses of *Arabidopsis thaliana* mutants depleted of FtsH metalloproteases and characterization of three FtsH6 deletion mutants exposed to high light stress, senescence and chilling. *New Phytologist* **191**, 449–458.
- Watanabe M, Balazadeh S, Tohge T, Erban A, Giavalisco P, Kopka J, Mueller-Roeber B, Fernie A, Hoefgen R.** 2013. Comprehensive dissection of spatio-temporal metabolic shifts in primary, secondary and lipid metabolism during developmental senescence in arabidopsis. *Plant Physiology* **162**, 1290–1310.
- Wittenbach VA.** 1978. Breakdown of ribulose biphosphate carboxylase and change in proteolytic activity during dark-induced senescence of wheat seedlings. *Plant Physiology* **62**, 604–608.
- Wittenbach VA, Lin W, Hebert RR.** 1982. Vacuolar localization of proteases and degradation of chloroplasts in mesophyll protoplasts from senescing primary wheat leaves. *Plant Physiology* **69**, 98–102.
- Wulster GJ, Sacalis JN, Janes HW.** 1984. Development of cyanide-resistant respiration in carnation flower petals. *Journal of the American Society for Horticultural Science* **109**, 202–206.
- Xia T, Xiao D, Liu D, Chai W, Gong Q, Wang NN.** 2012. Heterologous expression of ATG8c from soybean confers tolerance to nitrogen deficiency and increases yield in Arabidopsis. *PLoS ONE* **7**, e37217.
- Xiong Y, Contento AL, Bassham DC.** 2005. AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana*. *The Plant Journal* **42**, 535–546.
- Xiong Y, Contento AL, Bassham DC.** 2007a. Disruption of autophagy results in constitutive oxidative stress in Arabidopsis. *Autophagy* **3**, 257–258.
- Xiong Y, Contento AL, Nguyen PQ, Bassham DC.** 2007b. Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. *Plant Physiology* **143**, 291–299.
- Yamada T, Ichimura K, Kanekatsu M, van Doorn WG.** 2009. Homologs of genes associated with programmed cell death in animal cells are differentially expressed during senescence of *Ipomoea nil* petals. *Plant and Cell Physiology* **50**, 610–625.
- Yoshimoto K, Hanaoka H, Sato S, Tomohiko K, Tabata S, Noda T, Ohsumi Y.** 2004. Analysis of Arabidopsis ATG8 family proteins involved in autophagy, a vacuolar degradation process. *Plant and Cell Physiology* **45**, S110–S110.
- Yoshimoto K, Jikumar Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, Ohsumi Y, Shirasu K.** 2009. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. *The Plant Cell* **21**, 2914–2927.
- Zelisko A, Garcia-Lorenzo M, Jackowski G, Jansson S, Funk C.** 2005. AtFtsH6 is involved in the degradation of the light-harvesting complex II during high-light acclimation and senescence. *Proceedings of the National Academy of Sciences, USA* **102**, 13699–13704.
- Zelisko A, Jackowski G.** 2004. Senescence-dependent degradation of Lhcb3 is mediated by a thylakoid membrane-bound protease. *Journal of Plant Physiology* **161**, 1157–1170.
- Zientara-Rytter K, Lukomska J, Moniuszko G, Gwozdecki R, Surowiecki P, Lewandowska M, Liszewska F, Wawrzynska A, Sirko A.** 2011. Identification and functional analysis of Joka2, a tobacco member of the family of selective autophagy cargo receptors. *Autophagy* **7**, 1145–1158.