C. R. Biologies 333 (2010) 382–391

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Plant biology and pathology/Biologie et pathologie végétales

Senescence and death of plant organs: Nutrient recycling and developmental regulation

Sénescence et mort des organes végétaux : recyclage nutritionnel et régulation développementale

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ARTICLE INFO

Keywords:
Plant ageing
Leaf-senescence
Flowering
Nitrogen remobilisation
Autophagy
Target of Rapamycin

ABSTRACT

Senescence and programmed cell death are important features for plant development. By allowing nutrient recycling and reallocation all along plant life, senescence contributes to the plant survival and the developmental program. This review first presents the concept of senescence in the global whole-plant life story, with an emphasis on the control exerted by flowering. It then focuses on leaf-senescence and its control by hormones, nutrients and development. The role of autophagy and of the Target of Rapamycin (TOR) kinase as potential regulators integrating environmental and endogenous signals, which control cell proliferation, reprogramming and nutrient management, is finally considered.

Mots clés :
Vieillissement et sénescence végétale
Floraison
Remobilisation de l'azote
Autophagie
Kinase TOR

RÉSUMÉ

L'idée que la sénescence végétale ainsi que la mort cellulaire sont programmées et contrôlées par des facteurs endogènes est relativement récente. Cette revue discute, en outre, le concept de sénescence à l'échelle de la plante entière en focalisant sur le rôle de la floraison dans la durée de vie de la plante. Dans un second temps, la revue se focalise sur le processus de sénescence foliaire et le rôle des hormones végétales et du statut nutritif dans la régulation de l'apparition et de l'intensité des symptômes de sénescence. L'implication de l'autophagie et de la kinase *Target of Rapamycin* (TOR) dans l'adaptation des plantes à leur milieu et dans le contrôle de leur croissance et de leur mort est particulièrement discutée.

1. Introduction

Plants have many forms depending on reproduction strategies and lifespan. Annuals or biennials live for a few months only. These plants are usually monocarpic, flowering and giving seeds only once in their plant life. Perennials live many years and are usually polycarpic, producing seeds over several years. The maximal lifespan for polycarpic plants is 4600 years for the bristlecone pine tree (*Pinus longaeva*) and 3200 years for the giant sequoia (*Sequoia gigantea*). Ages over 10,000 years have been recorded for some clonal plants like Huckleberry (*Gaylussacia brachycerium*) [1]. It is assumed that plant death is the last step of a long process of senescence. If plant senescence is related to plant ageing, it is nonetheless noticeable that senescence symptoms can occur at different times during plant ageing and that the symptoms' severity is variable, depending on the environment and on genotypes. The concept of developmental senescence arose with the idea that all plant species and all genotypes

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doi:10.1016/j.crvi.2010.01.016

do not senesce and die at the same time and in similar ways and that death is actively induced by endogenous factors specific to each plant. Developmental senescence distinguishes thus between natural and accidental death [2]. Beside the senescence of the whole-plant that refers to the death of the individual, organ senescence and, more especially, leaf-senescence are also developmentally controlled. Leaf-senescence is accepted as a highly regulated, active process that can lead to programmed cell death (PCD). Developmental leaf-senescence occurs even when the plant is grown in good conditions (sufficient nutrition, optimal temperature and photoperiod), away from pathogen attacks and free of abiotic stresses such as darkness or drought. According to leaf age, developmental senescence is eventually initiated and will thereafter progress in a leaf age-dependent manner. It is, therefore, genetically programmed within the timetable of plant development [3].

While the two principal patterns of whole-plant death are monocarpy and polycarpy representing, respectively, a single reproductive phases followed by death and repeated reproductive phase followed by death, individual leaf-senescence is not obligatorily linked with the plant reproductive program. Of course, a complex organism like a plant must coordinate and integrate the activity of all its parts and the senescence of each part is certainly coordinated by a whole-plant program rather than being anarchic. Senescence and death of the different organs are intimately associated with the fact that plant growth is relatively undetermined compared to animals, with new organs appearing throughout the plant life.

In this article, we will review different aspects of developmental senescence from organ senescence to cellular cell death with a special focus on signals, regulatory mechanisms and structural responses included in the program or timetables of events leading to death.

2. Whole-plant senescence

The major developmental change in the plant life cycle is the initiation of flowering. Cessation of vegetative growth and the onset of flowering, with its effects on plant lifespan, are major components of whole-plant senescence of monocapic species even if they do not cause plant death in themselves. The decline in vegetative growth, particularly of new leaves, is reflected in the meristematic activity of apical meristems and cambium [1]. A consequence of decreasing cambial activity is a reduction of phloem activity and, as a result, impaired nutrient distribution. The cessation of vegetative growth is related to the conversion of the shoot apex to reproductive growth [4]. Preventing flowering might then allow monocarpic plants to reach a greater size and age than normal. The early experiments performed to explore the relationship between leaf-senescence and flowering were conducted by defruiting. It was shown that defruiting mignonette (*Reseda lutea*) and Vienna wallflowers (*Leucoium luteum*) restored vegetative growth. Mignonette, which is a small herbaceous plant, can then become a small tree.

Cessation of vegetative growth followed by flowering and fruiting is known to induce major changes in plant metabolism and nutrient redistribution and partitioning. A decrease in nitrogen uptake at the reproductive stage has been reported for rapeseed [5]. The growth of fruits and seeds represents a new sink that competes with the rest of the plant for nutrients. It was then proposed that in annual, biennial and other monocarpic plants like bamboo, sink strength of the fruits would drive death by exhausting the plant [6]. Therefore, defruiting could postpone death and senescence by preventing some of the metabolic declines normally associated with monocarpic senescence. However, this is probably not the only reason for this delay in developmental senescence. Whereas some sterile mutants show prolonged leaf greenness and photosynthesis compared to wild type (Fig. 1), there may be some large differences among species and all the sterile mutants do not show delayed senescence.

In contrast to annuals, redistribution of nutrients within the plant, accompanied by building carbon and nitrogen reserves and resource management may be the main components of plant longevity in perennials and especially in trees. Nevertheless, factors like the distances between shoot apices and roots in woody plants, the nutrient deficiency due to an increase in sink/source ratio with ageing the accumulation of minerals and salts or of other presumably toxic compounds might cause the death of polycarpic plants.

Another way to investigate the role of flowering in plant longevity was to inhibit flowering by avoiding the

Fig. 1. Leaf-senescence is delayed in sterile mutant (on the left) compared to the wild type (on the right).

vernalisation step that is required by plants like cabbage and sugar beet. Preventing flowering in this way allowed such annuals to live more than two years. From such observation, using Arabidopsis as a plant material for genetic analysis, Rick Amasino's group identified the *FLC* gene involved in the regulation of floral induction by vernalisation [7]. *FLC* prevents flowering in biennials unless they have experienced the cold of winter. Exposure to cold promotes flowering in biennials by a stable epigenetic switch of *FLC* to a repressed state. This epigenetic state of *FLC* is reset to an active state in the next generation [7]. Many ecotypes of Arabidopsis are extremely late flowering unless vernalised. In addition to the *FLC* locus, they contain a second locus named *FRIGIDA* (*FRI*) responsible for late flowering. *FLC* delays flowering in a rheostat-like manner. The delay in time to flowering in a *FRI*-containing background is proportional to *FLC* copy number. Transgenic plants containing an additional copy of *FLC* do not flower in the absence of cold treatment and thus do not senesce and behave like true biennials. In the annual vernalisation-independent accessions, the late-flowering allele of *FRI* is absent and the autonomous pathway down-regulates *FLC*. Recently, Wingler et al. [8] examined the genetic basis of sugar-regulated senescence (see below) using Arabidopsis recombinant inbred lines. Interestingly, these authors found flowering-dependent and -independent quantitative trait loci (QTL). The major flowering–dependent QTL co-localized with the *FRI* allele on chromosome 4. This work showed that whole-rosette senescence is genetically linked to the vernalisation-dependent control of flowering, and also controlled by flowering-independent pathways.

3. Leaf-senescence

Leaf-senescence is an active and regulated degeneration process that seems basically governed by the developmental age and has been evolutionarily selected. Leaf-senescence is an important phase in the leaf lifespan period that can last as long as leaf maturation (Fig. 2). During leaf-senescence, cells undergo rather orderly changes in cell structure, metabolism and gene expression [9]. The earliest and most significant change in cell structure is the chloroplast breakdown. Chloroplast contains up to 70% of the leaf proteins and most of the metabolic enzymes involved in photosynthesis, photorespiration, nitrogen assimilation, and amino acid biosynthesis. Metabolically, carbon and nitrogen assimilations are replaced by the catabolism of chlorophyll and macromolecules such as proteins, RNA and membrane lipids. The main function of leaf-senescence is to recycle cellular material accumulated during leaf growth and maturation into reusable and exportable nutrients to supply sink organs. Thus, leaf-senescence can be understood as a recycling process that contributes to a better nutrient management leading to an efficient resource economy for production of new organs and plant fitness. In naturally senescing leaves, senescence occurs in a coordinated manner, at the wholeleaf level starting from the tip and margins toward the base of the leaf. Leaf-senescence can occur without obvious correlation with senescence of other plant organs. Leaf sequential senescence refers to the competition for resources between lower leaves and younger foliage. Leaf sequential senescence is not obligatorily related to flowering [8] and can be observed during the vegetative stage. In annuals, the severity of sequential leaf-senescence symptoms is usually increased due to strong sink strength exerted by fruits and seeds. It is then considered that sequential leaf-senescence is boosted after flowering and the term "monocarpic leaf-senescence" used in this case by some authors refers to the symptoms observed after flowering. This can be somehow confusing according to the "whole-plant monocarpic senescence" that refers to plant death as explained above. In perennials, autumnal leaf-senescence of deciduous tree is certainly a consequence of diminishing day length and temperature. However, in that case also, source to sink nutrient transfer is the result of this developmental phase. Indeed, thanks to nutrient remobilisation from leaves, trunks are storing organic nitrogen, minerals and sugars that will help revival at spring. Since they largely determine yields, product quality and plant adaptation to environment the metabolic, physiological and regulation aspects of leaf-senescence are of considerable agronomical interest [10]. One important issue for fundamental research on leaf-senescence, as well as for agricultural challenges, is to determine when leaf-senescence starts and how long it lasts before the leaf dies. The duration of the process and the tight coordination of its onset with sink development are important for nutrient remobilisation efficiency and therefore for grain filling in crops. The molecular regulation of leaf-senescence has thus been extensively studied. A large number of genes that are up-regulated during senescence, called senescence-associated genes (*SAGs*), have been isolated from various plant species [11,12,9]. The expression and the regulation of some *SAG* genes like *SAG12* and *SAG13* is commonly monitored to determine leaf-senescence onset and progress.

A particularly important issue is to identify signals, regulatory mechanisms and genes that induce leaf-senescence. This question is complicated by the fact that in some plant species, like tobacco leaf, senescence is

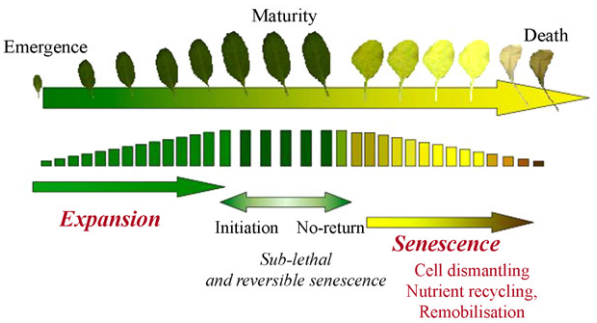

Fig. 2. Schematic representation of the main phase of leaf life story including leaf expansion, maturity and senescence. By definition, leaf maturity starts when leaf expansion is over and ends with the first senescence symptoms. During the maturity phase, the leaf is facing numerous sub-lethal events leading to many chronic senescence syndromes and recovery events. Leaf-senescence no-return syndrome is characterised by a succession of degradation process that will lead to death.

reversible, thereby hampering the detection of symptoms such as leaf yellowing. Leaf-senescence reversibility suggests that there is a time window of an adaptative senescence phase during which senescence-promoting factors and longevity factors compete, thus leading to a succession and alternation of sub-lethal chronic senescence symptoms and recoveries (Fig. 2). This may allow plants to respond and adjust to fluctuating environmental conditions. Moreover, genetic variation exists for symptoms of leaf-senescence and genotypes with leaves that remain green for longer than normal are termed *stay-green* varieties. During senescence, some of these genotypes or mutants retain both chlorophyll and photosynthetic competences (functional *stay-greens*) while others keep their chlorophyll but degrade some other component of photosynthetic apparatus (non-functional *stay-greens* or cosmetic *stay-greens*) [13].

4. Hormonal regulation and leaf-senescence

Hormonal control of leaf-senescence has been widely investigated (see [14] for a review). Cytokinin and ethylene have the most extensively documented roles in delaying or inducing leaf-senescence, respectively. Hormones that were reported to delay senescence are giberellic acid, auxin and cytokinin, i.e. hormones that promote growth. However, the exact effect of auxin and gibberelin remains variable and unclear [15]. Cytokinin has the strongest effect on leaf-senescence retardation [15]. The best demonstration of cytokinin effects was provided by the production of *SAG12::IPT* transgenic tobaccos [16]. Cytokinin synthesis by the *IPT* gene was enhanced when the *SAG12* promoter was induced. This resulted in a delay of leaf-senescence. The CKI1 (cytokinin independent 1) receptor and the Arabidopsis response regulator ARR2 appeared to be involved in regulating leaf-senescence [17]. The phosphorylation of ARR2 by AHK3 (Arabidopsis Histidine Kinase 3) is essential for controlling leaf longevity. Indeed the gain-of-function *ore12-1* mutant, carrying a missense mutation in *AHK3*, showed delayed leaf-senescence. By contrast, a loss-of-function mutation in *AHK3* conferred a reduce sensitivity to cytokinin in cytokinin-mediated delay of leaf-senescence (see [14] for a review). Recently, Balibrea Lara et al. [18] identified an extracellular invertase induced during cytokinin-mediated delay of senescence. When extracellular invertase is inhibited cytokinin can no longer inhibit leaf-senescence in transgenic *SAG12::IPT*. This suggests that cytokinin controls leaf-senescence through tuning sink/source leaf transition.

Hormones that induce leaf-senescence are abscissic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and ethylene. Under low nitrogen and high sugar conditions, the Arabidopsis *abi5* (*abscissic acid insensitive 5*) mutant showed delayed senescence. This is in good agreement with the role of sugar signalling during senescence (see below) since the b-ZIP transcription factor ABI5 can be induced by glucose and is increased during senescence [19]. The *abi2-1* and *abi1-1* mutants also presented early senescence symptoms when grown on low nitrogen with glucose [20]. Thus the onset of leaf-senescence induced by ABA may be coupled to metabolic signalling in Arabidopsis. Exogenous application of JA caused premature senescence in attached and detached leaves in wild-type Arabidopsis but failed to induce precocious senescence of JA-insensitive mutant *coi1* plants, suggesting that the JA-signalling pathway is required for JA to promote leaf-senescence [21]. SA is a key element in plant response to stress and pathogen attack. Evidence shows that SA has also a role in the regulation of genes during leaf-senescence [22]. More recently, Abreu and Munné-Bosch [23], by measuring the Fv/Fm ratio, uncovered that SA deciency in NahG transgenic lines and *sid2* mutants is associated with reduced damage to the photosynthetic apparatus compared to wild-type plants, and that SA influences plant growth, senescence and seed production. It is also well known that gaseous ethylene plays an important role in plant growth and development [24]. Exogenously applied ethylene induces premature leaf-senescence in Arabidopsis. The role of ethylene is demonstrated by functional analysis of mutants affected in the pathway. Ethylene insensitive mutants *etr1-1* and *ein2/ore3* showed increase in leaf longevity [25]. A large collection of *onset of leaf death* (*old*) mutants was isolated and showed that the effect of ethylene is limited to a range of leaf ages and that effect on leaf-senescence increases with leaf age [26,27]. The concept of senescence window arose from those observations and explained the age-dependent actions of hormones. This concept was developed from studies on the interactions between leaf age and ethylene but it can also be valuable for other hormones like cytokinins. The window concept assumes that leaf-senescence has three distinct development phases in relation to the permeation to hormone signalling. The first phase corresponds to early development and is a "never senescence phase". The second phase, that may correspond to the reversible phase in tobacco, is the window of adaptive and hormone-dependent senescence phase. Finally, the third phase is a hormone-independent and ineluctable senescence phase which happens even without the participation of hormones like ethylene ([25]; Fig. 3). Schippers et al. [14] proposed that the senescence window integrates the action of all plant hormones involved in leaf-senescence and proposed a tentative model illustrating the action of the different hormones during developmental senescence. Development is the primary regulator of senescence in this model. During ageing, developmental cues would lead to diminished action of senescence-retarding hormones such as auxin, gibbereline and cytokinin, as well as concomitant strengthening of the action of senescence enhancing hormones such as ethylene JA, SA and ABA (Fig. 3).

5. The role of regulatory factors in the control of leaf-senescence

Several regulatory factors which are involved in the control of leaf-senescence have been identified by screening early or late senescing mutants. For example, the *ORESARA9* (*ORE9*, oresara meaning "long-living" in Korean) gene encoding an F-box protein, that was isolated in 2001 by Woo et al. [28] as a regulator of leaf-senescence, was further identified as *MORE AXILARY BRANCHES2* (*MAX2*), a

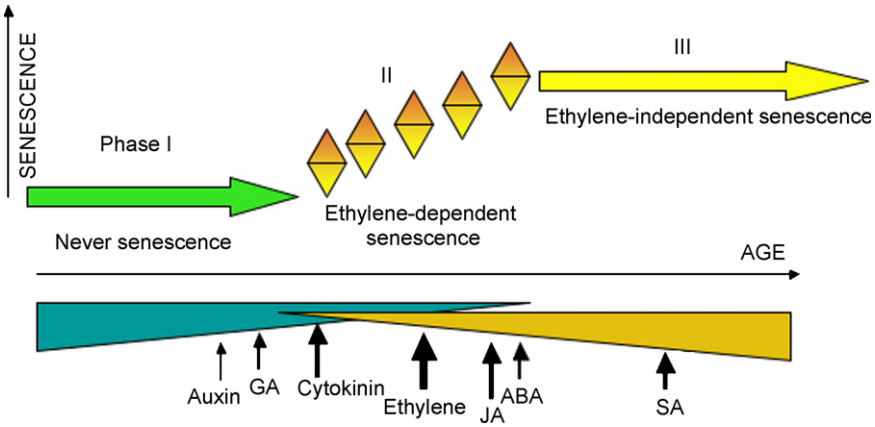

Fig. 3. The senescence window concept adapted from Jing et al. [25] and Schippers [14]. At early leaf development, ethylene is not able to induce leaf-senescence (phase I), permeation phase II allows senescence induction by ethylene; later, phase III presents senescence symptoms independently of ethylene action. The window of hormone-action depends on hormone type. The action of senescence-repressing hormones is decreasing with leaf age (blue triangle) while the action of senescence-promoting hormones increase (orange triangle). The different size arrows for hormones illustrate the respective importance of hormones in leaf-senescence control.

regulator of photomorphogenesis in Arabidopsis. *MAX2/ORE9* is therefore involved in inflorescence architecture and leaf-senescence. *MAX2* is expressed ubiquitously at the seedling stage and restricted to vascular tissue and meristems at adult stages [29]. MAX2 might regulate multiple targets at different developmental stages to optimize plant growth and development, including senescence. The Pea *MAX2* homologue has been identified as *RMS4*. The *rms4* mutants were further characterized as lacking a response to branching inhibition signal and to strigolactones [30]. It can be suspected that emergence and development of new sinks on mother plants of *max2* and *rms4* disturbed whole-plant metabolism and was the reason for the higher senescence of older organs and especially of older leaves.

ORE9 is not the only regulatory factor isolated using a genetic screen based on leaf yellowing symptoms. For example, *ORE1/AtNAC2* and *AtNAP*, which are NAC (NO APICAL MERISTEM (NAM), ATAF1, and CUP-SHAPED COTYLEDON2 (CUC2)) family transcription factors [31,32], have important roles in leaf-senescence. The miRNA are also involved in controlling leaf-senescence. Recently Kim et al. [32] provided evidence that *ORE1/AtNAC2* is positively regulated by *EIN2*, a component of the trifurcate pathway, that regulates other functions including ethylene signalling, cell growth control, and stress responses as well as leaf-senescence. *ORE1/AtNAC2* is also repressed by miR164 that target a group of NAC family genes (CUC1, CUC2, NAC1, ORE1, At5g07680, and At5g61430) and function in various developmental processes, including lateral root development and organ boundary formation in shoot meristem and flower development. The role of meristem genes in leaf-senescence is not specific for Arabidopsis. Expressing the maize meristem homeobox gene *KNOTTED1* under the control of a senescence–activated promoter has been shown to delay leaf-senescence [33].

The other way to identify transcription factors involved in leaf-senescence came from transcriptomic data analysis. A large set of transcription factors were found to be up-regulated in senescing Arabidopsis leaves [11,12,34]. Arabidopsis transcription factors have been grouped in around 20 different families, the largest groups being NAC, WRKY, C_2H_2-type zinc finger, AP2/EREBP and MYB proteins [14]. Among them WRKY53 was shown to play a central role in early leaf-senescence [35,36]. Indeed, WRKY53 knock down mutants displayed a delay in leaf-senescence while overexpressing WRKY53 induced precocious senescence. It was shown that WRKY53 targets were SAGs as well as other transcription factors involved in leaf-senescence.

6. Role of histone deacetylation in the epigenetic control of leaf-senescence

Different evidence suggested a role of epigenetic processes in leaf-senescence control. The role of histone deacetylation in leaf-senescence was documented in Arabidopsis by Tian and Chen [37] and Tian et al. [38]. Inhibiting histone acetylation by antisense suppression of *AtHD1* resulted in precocious leaf-senescence. In contrast, the global increase of H3 acetylation in *hda6* mutants and RNAi lines was correlated with an increase of leaf longevity and a delay in the expression of the senescence-associated *SAG12* and *SEN4* genes [39]. The *ORE7/ESC* gene that encodes an AT-hook DNA-binding protein was shown to modify the interphase chromatin organization and to affect initiation of leaf-senescence [40]. The recent report of Ay et al. [41] further showed that interphase chromatin organization is globally modified during leaf-senescence and that the histone H3K4 methylation modulated the transcriptional activation of WRKY53, a key regulator of leaf-senescence. During senescence, when the locus becomes activated, H3K4me2 and H3K4me3 are significantly increased at the 5′ end and in the coding region of this gene. Plant overexpressing the SUVH2 histone methyl transferase displayed delay in the expression of WRKY53 and other SAG genes as well as delay in leaf-senescence global symptoms [41].

7. C/N status, leaf-senescence and autophagy

In addition to hormones, there is evidence that the metabolic C/N status of the leaf serves as a general signal that can induce leaf-senescence and modulates the timetable of events. Light dosage influences leaf-senescence. While high light results in premature senescence, low light delays the senescence process [42]. Moderation of photosynthetic capacity that resulted from the *ore4* mutation delayed developmental senescence [43]. The role of sugars as a signalling molecule has been largely debated (see [44] for a review). Sugar feeding to whole-plant can induce leaf-senescence [45] and the manipulation of the sugar sensor hexokinase affects senescence. The *gin2-1* mutant in hexokinase 1(*HXK1*) shows delayed senescence [30] and reduced response to glucose feeding [46]. By contrast, overexpression of *HXK1* in the tomato accelerates leaf-senescence [47]. Nitrogen limitation, which is another factor that triggers sugar accumulation in leaves [48], induces early leaf-senescence symptoms [49–51]. Wingler et al. [51] showed that when glucose was added to low-nitrogen medium, leaf-senescence was clearly accelerated compared to plants grown under low-nitrogen medium without glucose. These authors suggested that addition of glucose might have accelerated the plant nitrogen utilization and led to a faster N-depletion from the medium, and as a result to an increased N-remobilisation. This study clearly pinpoints the role of the carbon/nitrogen balance in the regulation of leaf-senescence.

Interestingly, links between carbon and nitrogen availabilities, leaf-senescence and autophagy, were established by Hanaoka et al. [52]. Autophagy is an intracellular process that serves for the vacuolar degradation of cytoplasmic components (Fig. 4). The molecular machinery operating during autophagy was elucidated at the cellular level in yeast and mammals before plants (see [53] for a review). In animals and yeast, autophagy is a regulated process that allows the removal of damaged proteins and organelles and therefore participates to cell longevity. Beside its role in waste management and cell lifespan, autophagy in animals and yeast is also named type II cell death. Indeed, under some circumstances autophagy is an alternative cell death pathway to apoptosis (type I cell death) in animals and yeast ([54]; Fig. 4A and B). Alternatives between apoptosis and autophagy in animals would be controlled by Beclin1 (also named *ATG6* in plant) /Bcl2 interactions. Plant *atg6* mutants are lethal and ATG6 is involved in the PCD associated with the hypersensitive and innate immune responses [55,56]. Autophagy is involved in the immune response of plants to pathogens. Autophagy is also involved in leaf longevity under nutrient-limiting conditions (Fig. 5). Mutations in several autophagy genes, such as Arabidopsis *AtATG7*, *AtATG8*, *AtATG9* and *AtATG5,* induce early leaf yellowing symptoms and expression of senescence markers like *AtSEN1* in the older leaves. The leaf yellowing symptoms triggered by the *ATG* mutations are age-dependent and early senescence is only observed on the oldest leaves of rosettes. Recently, it has been demonstrated that autophagy is required for the senescence-dependent degradation of Rubisco [57]. It has also been suggested that autophagy has a role prior and during leaf-senescence for nutrient remobilisation and waste removal like in animal cells [44]. In animals, the control of autophagy by nutrient availability is mediated by the Target Of Rapamycin (TOR) kinase, a global integrator of environmental and endogenous signals that regulates growth and development (Fig. 4; see below).

8. Leaf-senescence, autophagy and the Target of Rapamycin signalling pathway

Multicellular organisms usually start as single cells, which grow, divide and develop into different organs. Unlike animals where the development plan is determined (i.e. growth is limited and the organization plan is conserved between individuals) and generally occurs in the embryo, plants start as simple organisms and develop new organs throughout their life. Therefore their development is highly plastic and responds to environmental signals to adjust to variations in nutrition, light, temperature, etc. As stated above, the plant relatively undetermined growth implies a continuous production of new organs and a concomitant loss of old parts through senescence and death. One can then see the life of a single leaf as the life of an independent organism, from birth to maturity and to death. Information recently gained from other organisms, mainly yeast and animals could shed new light on the mechanisms governing leaf-senescence.

Indeed, it has been recently shown that the TOR kinase controls lifespan in the worm *C. elegans*, in Drosophila and in mice [58,59]. In the last example, the administration of rapamycin, a molecule that specifically inhibits TOR, was found to extend lifespan of treated mice, as does caloric restriction in all these organisms. The TOR signalling pathway is conserved from yeast to man and plants and is involved in many cellular processes, including growth control, nutrient sensing and stress responses [60]. Indeed, in eukaryotic cells, the TOR kinase complexes are the nexus of the connections between the perception of external information like nutrient availability and growth-promoting processes (metabolism, protein translation, cytoskeleton organization etc). In brief, when conditions are favourable, the TOR kinase is activated and stimulates cell metabolism and growth. On the other hand, when external conditions are unfavourable (lack of nutrient, abiotic or biotic stresses, etc), the TOR kinase activity is inactivated which induces autophagic recycling, entry in the stationary phase (G0) in yeast and represses growth. In yeast and animal cells, TOR exists in at least two large protein complexes that serve in recruiting the various substrates of TOR. The first complex includes the TOR, RAPTOR/KOG1 and LST8 proteins and is sensitive to rapamycin. The second complex, containing TOR, RICTOR/AVO3 and LST8, phosphorylates Akt/PKB [60]. There are several lines of evidence for the existence of the TORC1 complex in plants but the TORC2 complex may be absent. Mutations which increase lifespan in yeast, *C. elegans* or Drosophila often affect components of the TOR and nutrient-signalling pathways [58]. Moreover, autophagy processes (Fig. 4) seem to be, at least partly, involved in the mechanisms prolonging life. It has also been shown that inhibition of TOR activity (or caloric restrictions) increases the activity

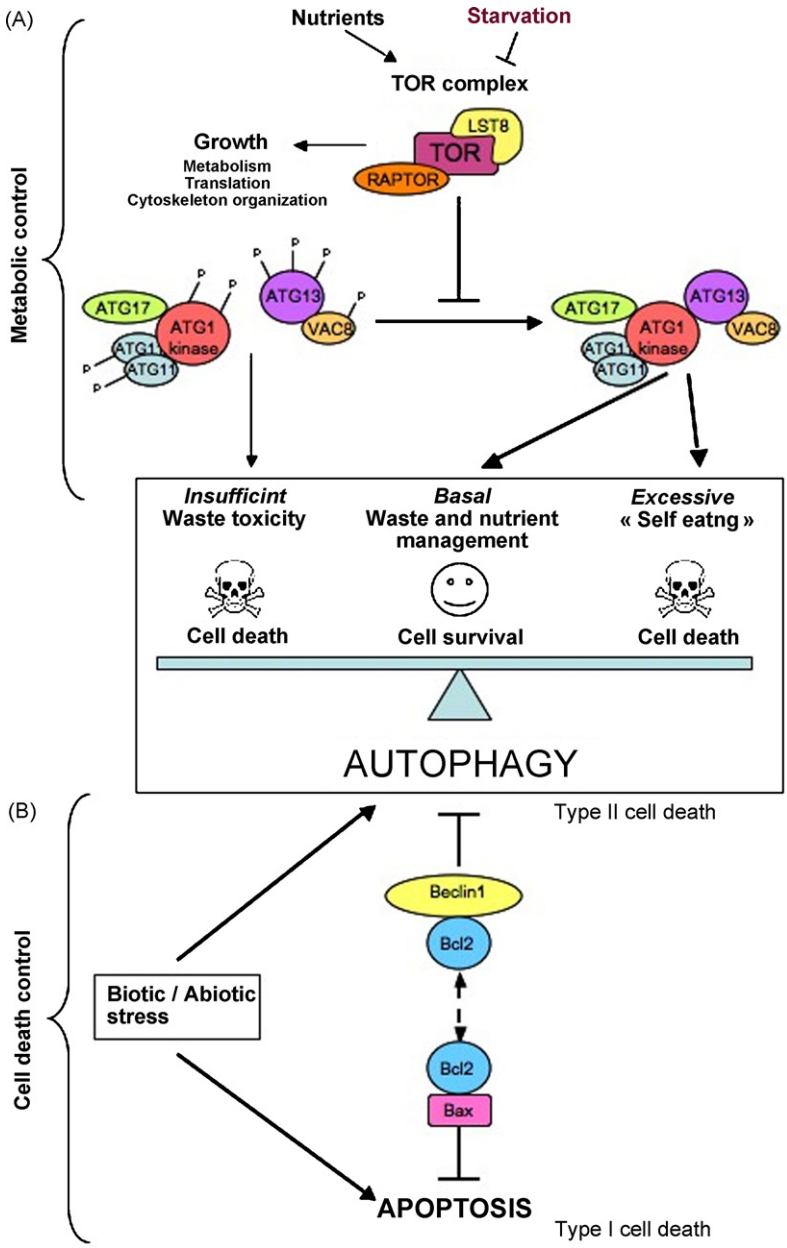

Fig. 4. Autophagy fine tuning determines cell survival and cell death. Basal autophagy acts as a cell survival mechanism through managing cells waste and provides metabolic substrates during nutrient deprivation. Excessive autophagy leads to cell death by self-eating. Insufficient or defective autophagy allowing damage proteins and organelles accumulation, results also in cell death. (A) Phosphorylation of ATG1, ATG13 and other proteins (ATG17, ATG11, VAC8) controls autophagy induction. TOR functions in a complex with RAPTOR and LST8 as a nutrient sensor and controls cell metabolism and growth. In nutrient-rich condition, TOR inhibits autophagy by phosphorylating ATG1 and ATG13. Phosphorylation leads to the dissociation of ATG13/ATG1 regulatory complex. In starvation condition, TOR is inhibited. Dephosphorylated ATG1 and ATG13 associate in a complex that mediates autophagy. (B) Autophagy (type II) and apoptosis (type I cell death) are controlled by the Bcl2 protein family in animal cells. Association between Bcl2 and Beclin1 (ATG6 homologue) inhibits autophagy in animal leading to apoptosis while the association between Bcl2 and Bax inhibits apoptosis allowing autophagy. Until now there is no evidence for the existence of Bcl2 or Bax in plants.

of sirtuins, a family of NAD+-dependent deacetylases [61]. The observation that fast-growing organisms (presumably having a highly activated TOR pathway) senesce and die early, supports the correlation between the level of activity of the TOR kinase and lifespan.

Plants have a specific role for senescence since they have the ability to recycle and reuse nutrients and components from senescing organs to support the growth of new parts (or the growth in the following year for annual plants). Unlike yeast and animals, it seems that the lack of macro-nutrients like nitrogen promotes rather than inhibits senescence and shortens rather than extends lifespan in plants. Conversely, as indicated above, low light or the lack of photosynthesis products (sugars) seems to

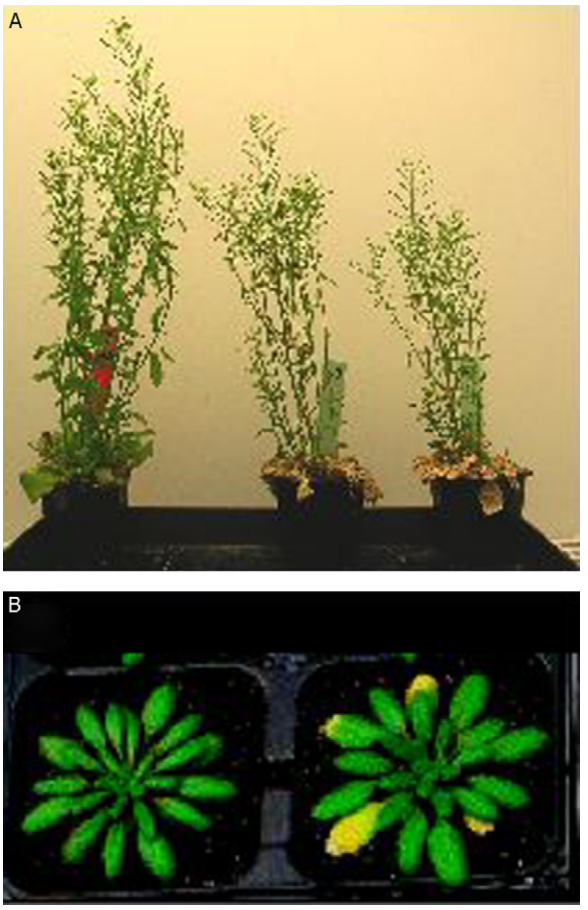

Fig. 5. (A) The silencing of the *AtTor* gene induces leaf yellowing and symptoms of early senescence in Arabidopsis (two independent silencing lines are shown on the right compared to a control Col0 Arabidopsis line on the left, Deprost et al., [62] for details). (B) RNAi silencing of the *AtAtg18a* gene induces leaf yellowing and symptoms of early senescence in Arabidopsis grown at low nitrate supply (on the right compared to a control Col0 Arabidopsis line on the left, *AtATG18a* RNAi line from Dr D. Bassham).

delay senescence in some cases. This may be due to the fact that plants, as autotrophic and immobile organisms, cannot rely on external and remote mineral feeding sources and have, therefore, the necessity to recycle their own components to either reproduce or develop new organs when resources are scarce.

We have previously identified and characterized a TOR gene ortholog (*AtTOR*) in Arabidopsis [62]. Loss of function of the *AtTor* gene was found to be embryo lethal and *AtTOR* expression was higher in young, developing organs. By overexpression and RNAi silencing, it was shown that decrease or increase in *AtTOR* expression modified organ and cell size, seed production and resistance to osmotic stress [63]. Interestingly, the conditional silencing of *AtTOR* led to a reduced and altered growth as well as to premature senescence symptoms, including yellowing of leaves linked to chlorophyll breakdown (Fig. 5). Leaves from these plants also accumulated very high amounts of soluble sugars (several hundred times more sucrose than in the control line, for example). Furthermore, we measured two- and three-times more glutamine synthetase and glutamate dehydrogenase activities, respectively, in leaves from silenced plants. These variations in enzyme activities and the soluble sugar level are usually associated with leaf-senescence and nutrient remobilisation in Arabidopsis [64]. This suggests that *AtTOR* activity is needed to restrain senescence and nutrient recycling. Moreover, an induction of the *SAG12* and other senescence marker genes was also observed (D. Deprost, unpublished results). This is consistent with the fact that the depletion of nutrient, which is supposed to decrease the TOR complex activity, causes plant senescence. The TOR kinase could thus play, like in yeast and animal cells, a central role in nutrient-signalling as a sensor of the nutritional and developmental status of the plant and regulate fundamental aspects of plant development like organ senescence (Fig. 4).

As stated above, autophagy is a well-known process in yeast and animals but it has only been recently established in plants (see [53,65] for reviews). Autophagy has indeed been widely investigated in animal and yeast because this process, through managing the wastes of the cells, has a role in increasing lifespan and decreasing cancers and other age-related diseases and appeared to be essential for cells longevity under nutrient-limiting conditions. In yeast and animals, autophagy is mainly controlled by the TOR kinase activity. In yeast, TOR inactivation regulates autophagic processes, and more specifically the formation of autophagosomes, by promoting the association between ATG1, a kinase which is a main regulator of autophagy, and ATG13, a regulatory subunit of ATG1. In nutrient-rich conditions, TOR probably hyperphosphorylates both ATG1 and ATG13, which in turn regulates the composition of the ATG1/ATG13 complex [66].

The way TOR and other nutritional and hormonal signalling pathways regulate the onset of senescence and nutrient recycling in plant organs remains to be determined. This will certainly produce exciting new information on the way plants adapt to their environment. Moreover, the knowledge gained from this field of research can provide new molecular tools for the improvement of crop yield through a greater optimization of nutrient recycling.

9. Root and flower senescence regulation

By contrast with leaf-senescence, root and flower senescence is less-well documented. Similar to leaf-senescence, flower senescence seems mainly related to ethylene and to nutrient accessibility [67]. It is, however, interesting to note that, unlike leaves, the senescence of flowers and roots is correlated with a lack of carbohydrates [67]. The role of autophagy in petal senescence and death was examined [68] and treatments using 3-methyladenine, an inhibitor of autophagy, resulted in reducing the time to visible petal senescence. Autophagy and TOR appear to be key elements in the control of petal, root and leaf developments that need to be better understood.

10. Conclusion

While senescence and even death is usually defined in different ways for plants and animals, the finding that the same genes can regulate basic cellular mechanisms involved in cell death in both types of organisms is asking questions about evolutionary processes and pressures that were operating during evolution of plant and animal organisms. An increasing amount of evidence suggests that metabolism and reproduction play a central role in regulating organ and organism senescence in both animals and plants. For instance, it is amazing that, in mice as in annual plants, avoiding reproduction increase lifespan [69]. Furthermore, it was shown that limiting sugar availability (caloric restriction in mammals, dark treatment on the whole-plant) extends lifespan [44]. Besides similarities, fundamental differences exist, of course, between plants and animals. Plant organs, like leaves, can senesce, die and be lost without affecting the survival of the whole-plant, which can be considered as an adaptation to nutrient limitation. Indeed, plants are immobile and must face nutrient limitations using altruistic behaviour like sacrificing and recycling parts and organs for the benefit of the rest of the organism.

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