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Research review paper

Molecular genetic control of leaf lifespan in plants - A review

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Abstract
Leaf senescence constitutes the last stage of leaf development in plants and proceeds through a highly regulated program in order to redistribution of micro- and macro-nutrients from the senescing leaves to the developing/growing plant organs. Initiation and progression of leaf senescence is accompanied by massive sequential alterations at various levels of leaf biology including leaf morphology and physiology, cell metabolism and structure, and gene transcription. In this regard, comprehensive expression analysis of senescence-associated genes *(SAGs*) and the identification of leaf senescence related mutants has revealed that leaf senescence is a complex genetically controlled program. In this review, we present important findings about the molecular genetic mechanisms underlying leaf senescence in various plants with a main focus on the model plant *Arabidopsis thaliana.* Functional analysis of leaf senescence mutants has provided new insights into the key processes that regulate the onset and progression of leaf senescence, thus allowing categorization of the various regulatory factors into several signalling pathways.

Keywords: Leaf senescence, longevity, *Arabidopsis.*

Introduction

Plant leaf senescence, as the last part of leaf development, is a type of programmed cell death (PCD) that takes place through an active and highly regulated process. The phenotype of leaf senescence is accompanied by a wide range of remarkable sequential changes at the level of cellular physiology, structure, metabolism and gene expression (Lim and Nam, 2005; Buchanan-Wollaston *et al*., 2005). It can also be modulated by various internal and external factors participating in modulating the senescence process. Internal factors mainly refer to the developmental ageing processes, reproduction and changes in levels of plant growth regulators (Schippers *et al*., 2007). While external elements include darkness, temperature, mineral deficiency, drought, oxidative stress and pathogen attack (Navabpour *et al*.,

2003; Pontier *et al*., 1999; Quirino *et al*., 1999). Therefore, incorporation of age dependent signals together with those factors decides the initiation time of leaf senescence and its succession rate (Lim *et al*., 2007). One of the important consequences of leaf cells degeneration is effective remobilization of transportable nutrients into developing tissues in aging plants (Lim *et al*., 2007) (Figure 1). In fact, leaf senescence marked by such a massive recycling of nutrients is essential for ensuring survivability of a species (Schippers *et al*., 2007). Hence, leaf senescence contributes to plant fitness and ensures survival of species in the following season by optimal production of offspring.

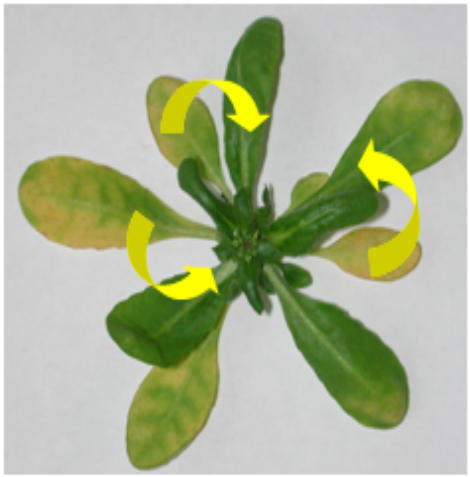

Figure 1. Representation of a senescing *Arabidopsis* plant showing the leaves at various ages and senescence levels. As leaves senesce, nutrients are relocated to other parts of the plants such as developing leaves and flowers.

Leaf senescence is a genetically controlled program

Initiation of developmental leaf senescence is accomplished by time-specific action of age-related genes (Schippers *et al*., 2007). As expected, the gene expression profiles of ageing leaves are distinctively different from young leaves. Many of the genes expressed in green leaves are down-regulated (senescence genes, *SDGs*), while other genes are up-regulated (senescence-associated genes, *SAGs*) (Gepstein *et al*., 2003). Towards identification of the genes involved in the leaf senescence, three main approaches significantly improve our understanding concerning molecular genetic regulation of leaf senescence. The first approach includes comprehensive transcriptional profiling of *SAGs*. The second involves the characterization of mutants with altered regulation of leaf senescence. The third one refers to analysis of quantitative trait loci (QTLs) for developmental /stress-induced leaf senescence in plants (Luquez *et al*., 2006).

Identification of the genes that are differentially expressed before and during the process of leaf senescence has received considerable attention over the last decade. In general, senescence down-regulated genes (*SDGs*) are involved in anabolic activities, while up-regulated genes (*SAGs*) are mostly involved in catabolic functions (Kim *et al*., 2007). Buchanan-Wollaston *et al*. (2005) identified more than 800 *SAGs* demonstrating reproducible increase in transcript abundance during developmental ageing in *Arabidopsis* leaves using. Liu *et al*. (2008) identified 815 expressed sequence tags (ESTs) up-regulated genes at the onset of flag leaf senescence in rice. Among the

identified *SAGs* are those encoding enzymes for the implementation of the senescence syndrome (Chlorophyll and Lipid degradation, nitrogen remobilization) (Dangl *et al*., 2000), various proteases (Thompson and Vierstra, 2005), transcription factors (Buchanan-Wollaston *et al*., 2005) and receptors for senescence perception signals (Guo *et al*., 2004). Therefore, global expression analysis provides important clues for understanding the metabolic and regulatory systems associated with the leaf senescence syndromes.

To gain more insights into the mechanism(s) of leaf senescence and longevity, functional analysis of regulatory genes is necessary. One approach is to directly study mutants in which we categorize and discuss the known senescence mutants based on their probable timely involvement in leaf senescence process.

Mutants involved in perception and transduction of senescence signals

Transcription factor genes

Various transcription factor (TF) genes show enhanced expression in senescing leaves. Ninety-six up-regulated TF genes were identified in ageing leaves of *Arabidopsis* (Buchanan-Wollaston *et al*., 2005). Guo *et al*. (2004) identified 134 genes that encode various TFs categorized in 20 different gene families in *Arabidopsis*. NAC and WRKY proteins constitute a big fraction of the identified senescence-related TFs. Involvement of NAC TFs in leaf senescence was further illustrated in a knockout line carrying a T-DNA knockout insertion in *AtNAP* (*At1g69490*), which considerably delayed leaf senescence in *Arabidopsis* (Guo and Gan, 2006). Another ABA-responsive NAC TF has been recently identified and termed VND-INTERACTING 2 (*VIN2*), which is regulated by developmental aging leaf processes, in association with salt-induced stress in *Arabidopsis* (Yang, *et al*., 2011). Moreover, NAC factor ORE1 (also called ANAC092 and AtNAC2) is another crucial TF which triggers early senescence, by regulation of various *SAGs* in a downstream gene network, when enhanced its expression in Arabidopsis thaliana. Promoter–reporter (GUS) studies showed that leaf and flower senescence induced by salt stress also regulate *ORE1* expression levels (Rauf *et al*., 2013; Balazadeh *et al*., 2010). In addition to NAC TFs, a number of WRKY family TFs play vital functions in *Arabidopsis* leaf senescence regulation. Especially, AtWRKY53 and WRKY6 have been associated with leaf senescence. A T-DNA insertion in *WRKY53* induced retarded leaf senescence event in transgenic lines (Miao *et al*., 2004). It has been recently suggested that three WRKY TFs WRKY70 and WRKY54, as co-operated negative regulators of leaf senescence, as well as WRKY53 might contribute in a regulatory system which incorporate internal and environmental signals, perhaps through interaction with WRKY30, to adjust the initiation and succession of leaf senescence (Besseau *et al*., 2012).

Signalling molecules

Senescence is associated with up-regulation of various genes that are potentially implicated in signal perception and transduction. Receptor kinases are probably involved in perception and transduction of senescence signals through protein phosphorylation.

A senescence-associated receptor-like kinase (*SARK*) isolated from bean leaves has been exclusively expressed during senescence. Interestingly, light and cytokinin treatments delayed the induction of *SARK* transcript, while darkness and ethylene accelerated this process (Hajouj *et al*., 2000). Another possible signalling pathway involved in leaf senescence is correlated with the function of the *MAPK*s (Mitogen-activated protein kinases) (Hirt, 1997). High levels of *ZmMPK5* transcripts were detected in senescing leaves of maize suggesting that *ZmMPK5* plays a role in the coordinated process of leaf senescence (Berberich *et al*., 1999).

Developmental leaf senescence regulatory genes

Arabidopsis leaves have a definite lifespan and eventually initiate to senesce even under perfect growth circumstances (Jing *et al*., 2002). Hence, the leaf senescence initiation is mainly the consequence of age-related changes (ARCs). There might be a cellular mechanism(s) that measures the age of a cell, tissue, organ or whole body for initiation of leaf senescence (Lim *et al*., 2003). This poses several questions, for instance, what does control the leaf longevity and how is developmental age distinguished to initiate the senescence program. A number of genetic loci were identified through the interaction between age-related changes and ethylene action which are based on the fact that certain ARCs have occurred in the leaf before initiation of senescence (Shirzadian-Khorramabad *et al*., 2010b, Jing *et al*., 2002). The observations can be summarized into the senescence window conception, which proposes three phases in the leaf development program based on the interaction between age-related changes and ethylene action (Jing *et al*., 2003). Mutations in genes acting at the three phases may result in predictable senescence phenotypes. This prediction was experimentally confirmed by the isolation and characterization of *onset of leaf death* (*old*) mutants (Shirzadian-Khorramabad *et al*., 2010a; Shirzadian-Khorramabad *et al*., 2010b; Shirzadian-Khorramabad *et al*., 2008, Jing *et al*., 2005; Jing *et al*., 2002). For instance, genes functioning at the first phase are the master regulators that incorporate the information from diverse sources and determine when and how senescence initiates. The *onset of leaf death 101 (old101)* mutant delayed initiation of leaf senescence and prolonged leaf longevity in both ethylene- and age-dependent leaf senescence manners (Shirzadian-Khorramabad *et al*., 2010b; Shirzadian-Khorramabad *et al*., 2008). Consequently, the first stage of ethylene senescence windows (Never senescence) may extend and shift further in *old101* plants (Shirzadian-

Khorramabad *et al*., 2008; Sturre *et al*., 2009). The genes working at the second phase presumably are those that govern the duration and speed of senescence. It was suggested that *OLD1*, *OLD2* and *OLD3* genes might regulate the first transition from the never-senescence phase to the ethylene-dependent phase (Shirzadian-Khorramabad *et al*., 2010b; Jing *et al*., 2002). At the last stage, there is "a point of no return" for senescence and cell death. Since *old101* plants normally start senescing later, it suggests that the switch to the third phase is delayed in *old101* plants (Shirzadian Khorramabad *et al*., 2008).

Metabolic rate and cellular redox balance play important roles in the regulation of developmental leaf aging. Identification of the *ore4-1* mutation supported involvement of metabolic rate and cellular redox balance in leaf senescence initiation (Woo *et al*., 2002). The late senescence phenotype of *ore4-1* plants is due to a decrease in metabolic rate, indicating that energy expenditure is a major factor in regulation of leaf senescence. Moreover, it was also suggested that the reduced metabolic rate results in a decrease in the generation of reactive oxygen species and therefore prolongs lifespan as found in the *ore4-1 Arabidopsis* mutants (Woo *et al*., 2002). In *old5* plants, increased oxidative stress is associated with alteration in the metabolite profile (Schippers *et al*., 2009). Thus, the damage generated by ROS can induce early leaf senescence onset, which is consistent with the 'free-radical' theory of ageing (Harman, 1956).

Mutants involved in hormone signalling

Leaf senescence could be modified by endogenous developmental factors such as phytohormones with cytokinin and ethylene having the most obvious effects on delaying or inducing leaf senescence. Here we utilize the availability of various mutants in the hormonal signalling pathways of cytokinin and ethylene to review how these hormones are involved in the regulation of the leaf senescence process.

Cytokinin

The endogenous cytokinin level drops during leaf senescence, and either exogenous application or endogenous enhancement of cytokinin content delays senescence (McCabe *et al*., 2001; Gan and Amasino, 1995). Molecular analysis at genomic scale revealed that genes involved in cytokinin biosynthesis and signalling, such as isopentenyl-transferase (*IPT*) gene is down-regulated. In contrast, the genes for cytokinin degradation and cytokinin oxidase, are up-regulated during leaf senescence (Buchanan-Wollaston *et al*., 2005). The gain-of-function *Arabidopsis* mutant, *ore12-1*, which has a missense mutation in *Arabidopsis Histidine Kinase 3* (*AHK3*) gene, exhibited a delay in leaf senescence (Kim *et al*., 2006). Interestingly, the loss-of-function *ahk3* mutant resulted in early senescence during dark-induced senescence suggesting that the effects of cytokinins in retardation of leaf senescence might

be through stimulation of the leaf photosynthetic activity (Schippers *et al*., 2007). Moreover, it was shown that the delay of senescence by cytokinin is mediated by an extracellular invertase (*Cin1*). Inhibition of extracellular invertase activity blocked cytokinin mediated retardation of leaf senescence (Balibrea Lara *et al*., 2004). The results further suggest that carbohydrate partitioning in association with extracellular invertase activity might be involved in cytokinin-mediated delay of leaf senescence.

Ethylene

Ethylene has been considered as an essential hormone in regulation of the leaf senescence initiation (Jing *et al*., 2002). Nine percent of the genes that are up-regulated during senescence are at least twofold reduced in the ethylene insensitive mutant 2 (*ein2*) (Buchanan-Wollaston *et al*., 2005). Ethylene insensitive *Arabidopsis* mutants *etr1-1* and *ein2/ore3* (Grbić and Bleecker, 1995) show increased leaf longevity. Both *ctr1* (constitutive triple response *1*) and ethylene-insensitive *etr1-1* mutant *Arabidopsis* plants grown in the continuous presence of exogenous ethylene did not accelerate senescence (Grbić and Bleecker, 1995). These results suggest that ethylene plays an important role in the dynamic coordination of the timely transition of a leaf to the senescence state (Jing *et al*., 2003; Grbic and Bleecker, 1995). Using an ethylene-induced senescence screen method, several *onset of leaf death* (*old*) mutants were identified through the interaction between leaf age and ethylene (Shirzadian-Khorramabad *et al*., 2010b; Jing *et al*., 2005; Jing *et al*., 2002) This suggests that multiple genetic loci are required to regulate the action of ethylene in leaf senescence. Therefore, it could be concluded that endogenous ethylene levels are important for the initiation of senescence, but ARCs limit its function within a specific age range (Shirzadian-Khorramabad *et al*., 2008).

Mutants involved in macromolecular degradation

Protein degradation

Degradation of proteins into amino acids and their subsequent remobilisation to developing organs in plants are considered as the essential steps during the leaf senescence. Up to 70% of the leaf proteins that are located within chloroplasts are degraded and remobilised during leaf senescence process. Currently a large set of genes involved in protein turnover, which are activated during plant senescence has been identified. Those include various proteases such as cysteine proteases, cathepsin B-like cysteine proteases, aspartic proteases as well as vacuolar processing enzymes and components of the novel autophagic pathway (Thompson and Vierstra, 2005; Gepstein *et al*., 2003). ClpD/ERD1 and ClpC1 proteases which are localized in chloroplast stroma may play the major regulatory roles in control of protein turnover in senescing leaves. Up-regulation of these two proteases might reflect the need for the recruitment of unfolded proteins for degradation by Clp proteases during senescence (Lin

and Wu, 2004). The housekeeping Clp proteases are involved in maintenance of appropriate stoichiometry and elimination of damages or mistargeted proteins (Adam and Clarke, 2002). An interesting class of genes that are up regulated during senescence encodes autophagy-related proteins (Buchanan-Wollaston *et al*., 2005).

Autophagy (self-eating), another protein cell degradation system in plant senescing cells, is an universal mechanism in eukaryotic cells involving in vacuolar bulk degradation of cytoplasmic components to recycle needed nutrients, degrade damaged or toxic components, or to reclaim cellular materials (Bassham, 2007). Three *Arabidopsis* autophagy proteins *AtAPG7*, *AtAPG9* and *AtATG18a* were found to have a function in the initiation of leaf senescence. Disruption of *Arabidopsis* genes *AtAPG7* and *AtAPG9* promoted leaf senescence, which implies that autophagy is necessary to stabilize cellular viability especially during situations that require substantial nutrient recycling (Hanaoka *et al*., 2002).

Chlorophyll degradation

Leaf senescence as the final step of leaf development is accompanied by leaf yellowing, which is a good indicator of senescence caused by chlorophyll (Chl) degradation (Matile, 2000). Chls in chloroplast thylakoid membranes are degraded to non-fluorescent Chl catabolites following their accumulations in the vacuoles of senescing cells (Hörtensteiner, 2009). Chls in higher plants consist of Chl *b* and Chl *a*, which is the most important compound of the photosystem I (PSI) and photosystem II (PSII) reaction centre complexes. Degradation of Chl *a* and Chl *b* has some overlapping steps. First, Chl *a* is converted into chlorophyllide *a* (Chlide *a*) by chlorophyllase, whose activity is present in the inner envelope chloroplast membranes (Matile *et al*., 2000). Chlide *a*, which considers as the last green compound in the chlorophyll breakdown pathway, is then converted into another compound termed pheophorbide *a* (Pheide *a*). Pheide *a* is subsequently converted into red Chl catabolite (RCC) by oxygenase (PAO). RCC is then catabolised into primary fluorescent chlorophyll catabolites by RCC reductase (RCCR) (Hörtensteiner, 2009). Degradation of Chl *b* starts with Chl *b* conversion into Chl *a* following the Chl *a*-degrading pathway as already described in the above three phases. Regarding diverse steps of Chl catabolism, several “stay green” or “non-yellowing” mutants were isolated from various plants, and their subsequent characterizations opened new insights into the genetic and biochemical mechanisms of Chl breakdown during leaf senescence (Hörtensteiner, 2009). Some stay green mutants can photosynthesize longer time and might therefore be expected to give a higher yield; they can be defined as ‘functional stay green’ mutants (Shirzadian-Khorramabad *et al*., 2010b), which have the potential to enhance plant productivity.

Identification of NON-YELLOW COLORING1 *NYC1* from rice (Kusaba

et al., 2007) supported the assumption that reduction of Chl *b* to Chl *a* within the Chl-protein complexes of the photosystems (PSI and PSII) is prerequisite for Chl degradation, leading to the destabilization of these Chl-protein complexes (Hörtensteiner, 2009). The stay green mutants *nyc1* preserves granal structure during senescence, suggesting that degradation of LHCII (Light-harvesting complexes of II) is required for proper degeneration of the thylakoid membrane during senescence.

Another group of stay-green mutants having a defection in the gene called *SID* (*senescence-induced degradation*). *SID* orthologous genes are annotated in Rice SGR (STAY-GREEN)] (Jiang *et al*. 2007) and in Arabidopsis as [*SGN/NYE1(STAYGREEN/NONYELLOWING*)] (Ren *et al*. 2007).

Lipid degradation

Degradation of membrane lipids is not just a symptom of the senescence process, but it is involved in regulation of age-dependent senescence progression (He and Gan, 2002). The membrane degradation is considered as a significant decline in amount of the phospholipids and relative enhancement of sterols and free fatty acids in the cell membrane (Manoharan *et al*., 1990) that eventually leads to the loss of membrane structural integrity (Thompson *et al*., 1998). Up-regulation of several genes involved in degradation of membrane lipids has been reported during senescence or after exposure to stress (Bargmann and Munnik, 2006). Plant cells contain various phospholipid-degrading enzymes such as D, C and A types of phospholipases and nonspecific acyl hydrolases, and lipoxygenases (Thompson *et al*., 1998; Fan *et al*., 1997). The significance of these enzymes in leaf senescence was investigated through functional analysis of *PLDα*, acyl hydrolase (*SAG101*) and lipases. Silencing of *SAG101*, which encodes an acyl hydrolase, leads to retarded leaf senescence in *Arabidopsis* (He and Gan, 2002). Ectopic expression of *SAG101* accelerated leaf senescence in young leaves. *SAG101* serves as a facilitating membrane breakdown in senescing leaf cells. Regulatory role of *PLDα* in hormone-induced leaf senescence were demonstrated in *PLDα*-antisense transgenic plants which showed delayed senescence (Fan *et al*., 1997), demonstrating that PLDα is an essential mediator in phytohormone-promoted leaf senescence.

Nutrient remobilisation

During the leaf senescence, the breakdown of leaf cell components is initiated at the chloroplast, where most of the nitrogen and other nutrients are stored. The massive breakdown and remobilisation is accompanied by up-regulation of *SAGs* encoding for hydrolytic enzymes, which are responsible for salvage of various macromolecules such as proteins, nucleic acids, polysaccharides and lipids (Buchanan-Wollaston *et al*., 2005). Moreover, genes encoding amino acid permeases and peptide transporters are also up-regulated to facilitate export from the senescing leaves (Buchanan-Wollaston *et al*., 2005). Leaf senescence

is associated with a drop in levels of various elements among which nitrogen was found to be the major recycled element (Himelblau and Amasino, 2001). In various plant species, remobilisation of nitrogen mostly occurs in the form of glutamine and asparagine (Finnemann and Schjoerring, 2000). During the senescence process, concentration of glutamine and asparagine increase in the phloem (Herrera-Rodriguez *et al*. 2006), implying central functions for glutamine and asparagine in providing N accessible for remobilisation from the dying leaves. Several approaches have identified many nitrogen transporter genes encoding for different amino acid transporters in seed and senescing leaves (Masclaux-Daubresse *et al*., 2008). Additionally, a positive correlation between the increase in proteolytic activity and the expression of three groups of senescence marker genes encoding for glutamine synthetase (GS) and glutamate dehydrogenase (GDH) have been reported (Terce-Laforgue *et al*., 2004). Overall, these results indicate that nutrient remobilisation during plant senescence is a complex process in which many regulatory systems play a role.

Perspectives

Leaf senescence is a complex genetically regulated program that contributes to plant fitness through remobilisation of the nutrients from ageing leaves to the developing organs. Understanding the various aspects of the regulatory mechanisms underlying this complex process has been an important task over the last decade. However, the entire picture of leaf senescence regulation remains obscure in biology. Therefore, new approaches are required to let us move ahead towards understanding all features of the leaf senescence regulation. In this respect, identification and functional characterization of novel senescence mutants can notably contribute towards this objective.

References

Adam, Z. and Clarke, A.K. 2002. Cutting edge of chloroplast proteolysis. *Trends Plant Sci.*, 17: 451-456.

Balazadeh, S., Siddiqui, H., Allu, A.D., Matallana-Ramirez, L.P., Caldana C., Mehrni,a M., Zanor, M.I., Köhler, B. and Mueller-Roeber, B. 2010. A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. *Plant* J. 62: 250-64.

Balibrea Lara, M.E.B., Garcia, M.C.G., Fatima, T., Ehness, R., Lee, T.K., Proels, R., Tanner, W. and Roitsch, T. 2004. Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. *Plant Cell*, 16: 1276-1287.

Bargmann, B.O.R. and Munnik, T. 2006. The role of phospholipase D in plant stress responses. *Curr. Opin. Plant Biol.*, 9: 515-522.

Bassham, D.C. 2007. Plant autophagy: more than a starvation response. *Curr. Opin. Plant Biol.*, **10:** 587-593.

Berberich, T., Sano, H. and Kusano, T. 1999. Involvement of a MAP kinase, ZmMPK5, in senescence and recovery from low-temperature stress in maize. *Mol. Gen. Genet.*, 262: 534-542.

Besseau, S., Li, J. and Palva, T. 2012. WRKY54 and WRKY70 co-operate as negative regulators of leaf senescence in *Arabidopsis thaliana*. *J Exp Bot.* 63: 2667-79.

Buchanan-Wollaston, V., Page, T., Harrison, E., Breeze, E., Lim, P.O., Nam, H.G., Lin, J.F., Wu, S.H., Swidzinski, J., Ishizaki, K. and Leaver, C.J. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signaling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. *Plant J.*, 42: 567-585.

Dangl, J., Dietrich, R. and Thomas, H. 2000. Senescence and programmed cell death. In Biochemistry and Molecular Biology of Plants (Buchanan, B., Gruissemand, W. and Jones, R., eds). Rockville, Maryland: American Society of Plant Physiologists, 1044-1100.

Fan, L., Zheng, S. and Wang, X. 1997. Antisense suppression of phospholipase D retards abscisic acid- and ethylene-promoted senescence of postharvest *Arabidopsis* leaves. *Plant Cell*, 9: 2183-2196.

Finnemann, J. and Schjoerring, J.K. 2000. Post-translational regulation of cytosolic glutamine synthetase by reversible phosphorylation and 14-3-3 protein interaction. *Plant J.*, 24:171-181.

Gan, S. and Amasino, R.M. 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. *Science*, 270: 1986-1988.

Gepstein, S., Sabehi, G., Carp, M.J., Hajouj, T., Nesher, M.F., Yariv, I., Dor, C. and Bassani, M. 2003. Large-scale identification of leaf senescence-associated genes. *Plant J.*, 36: 629-642.

Grbić, V. and Bleecker, A.B. 1995. Ethylene regulates the timing of leaf senescence in *Arabidopsis*. *Plant J.*, 8: 595-602.

Guo, Y. and Gan, S. 2006. AtNAP, a NAC family transcription factor, has an important role in leaf senescence. *Plant J.*, 46: 601-612.

Guo, Y., Cai, Z. and Gan, S. 2004. Transcriptome of *Arabidopsis* leaf senescence. *Plant Cell Environ.*, 27: 521-549.

Hajouj, T., Michelis, R. and Gepstein, S. 2000. Cloning and characterization of a receptor-like protein kinase gene associated with senescence. *Plant Physiol.*, 124: 1305-1314.

Hanaoka, H., Noda, T., Shirano, Y., Kato, T., Hayashi, H., Shibata, D., Tabata, S. and Ohsumi, Y. 2002. Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an *Arabidopsis* autophagy gene. *Plant Physiol.*, 129: 1181-1193.

Harman, D. 1956. Ageing: a theory based on free radical and radiation chemistry. *J. Gerontol.*, 2: 298-300.

He, Y. and Gan, S. 2002. A gene encoding an acyl hydrolase is involved in leaf senescence in *Arabidopsis*. *Plant Cell*, 14: 805-815.

Herrera-Rodriguez, M.B., Maldonado, J.M., Perez-Vicente, R. 2006. Role of asparagine and asparagine synthetase genes in sunflower (Helianthus annuus) germination and natural senescence. *J. Plant Physiol.*, 163: 1061-1070.

Himelblau, E. and Amasino, R.M. 2001. Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. *J. Plant Physiol.*, 158: 1317-1323.

Hirt, H. 1997. Multiple roles of MAP kinases in plant signal transduction. *Trends Plant Sci.*, 2: 11-15.

Hörtensteiner, S. 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence, *Trends Plant Sci.*, 14: 155-162.

Jiang, H., Li, M., Liang, N., Yan, H., Wei, Y., Xu, X., Liu, J., Xu, Z., Chen, F. and Wu, G. 2007. Molecular cloning and function analysis of the *stay green* gene in rice. *Plant J.*, 52: 197-209.

Jing, H.C., Hille, J. and Dijkwel, P.P. 2003. Ageing in plants: conserved strategies and novel pathways. *Plant Biol.*, 5: 455-464.

Jing, H.C., Schippers, J.H.M., Hille, J. and Dijkwel, P.P. 2005. Ethylene-induced leaf senescence depends on age-related changes and *OLD* genes in *Arabidopsis*. *J. Exp. Bot.*, 546: 2915-2923.

Jing, H.C., Sturre, M.J., Hille, J. and Dijkwel, P.P. 2002. *Arabidopsis* onset of leaf death mutants identify a regulatory pathway controlling leaf senescence. *Plant J.*, 32: 51-63.

Kim, H.J., Lim, P.O. and Nam, H.G. 2007. Molecular regulation of leaf senescence. In: Senescence processes in plants-Gan S, ed. Oxford: Blackwell Publishing. 231-255.

Kim, H.J., Ryu, H., Hong, S.H., Woo, H.R., Lim, P.O., Lee, I.C., Sheen, J., Nam, H.G. and Hwang, I. 2006. Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, 103: 814-819.

Kusaba, M., Ito, H., Morita, R., Iida, S., Sato, Y., Fujimoto, M., Kawasaki, S., Tanaka, R., Hirochika, H., Nishimura, M. and Tanaka, A. 2007. Rice NON-YELLOW COLORING1 is involved in light-harvesting complex II and grana degradation during leaf senescence. *Plant Cell*, 19:1362-1375.

Lim, P.O. and Nam, H.G. 2005. The molecular and genetic control of leaf senescence and longevity in *Arabidopsis*. *Curr. Top. Dev. Biol.*, 67: 49-83.

Lim, P.O., Kim, H.J. and Nam, H.G. 2007. Leaf senescence. *Ann. Rev. Plant Biol.*, 58: 115-136.

Lim, P.O., Woo, H.R. and Nam, H.G. 2003. Molecular genetics of leaf senescence in *Arabidopsis*. *Trends Plant Sci.*, 8: 272-278.

Lin, J.F. and Wu, S.H. 2004. Molecular events in senescing *Arabidopsis* leaves. *Plant J.*, 39: 612-628.

Liu, L., Zhou, Y., Zhou, G., Ye, R., Zhao, L., Li, Z. and Lin, Y. 2008. Identification of early senescence-associated genes in rice flag leaves. *Plant Mol. Biol.*, 67: 37-55.

Luquez, V.M.C., Sasal, Y., Medrano, M., Martın, M.I., Mujica, M. and Guiame, J.J. 2006. Quantitative trait loci analysis of leaf and plant longevity in Arabidopsis thaliana. *J. Exp. Bot.*, 57: 1363–1372.

Manoharan, K., Prasad, R. and Mukherjee, S. 1990. Senescence-related lipid change in callus cultures of *Datura innoxia*. *Phytochem.*, 29: 2529-2531.

Masclaux-Daubresse, C., Reisdorf-Cren, M. and Orsel, M. 2008. Leaf nitrogen remobilisation for plant development and grain filling, *Plant Biol.*, 10: 23-36.

Matile, P. (2000). Biochemistry of Indian summer: Physiology of autumnal leaf coloration. *Exp. Gerontol.*, 35: 145-158.

McCabe, M.S., Garratt, L.C., Schepers, F., Jordi, W.J., Stoopen, G.M., Davelaar, E., van Rhijn, J.H., Power, J.B. and Davey, M.R. 2001. Effects of P-*SAG12*:IPT gene expression on development and senescence in transgenic lettuce. *Plant Physiol.*, 127: 505-516.

Miao, Y., Laun, T., Zimmermann, P. and Zentgraf, U. 2004. Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. *Plant Mol. Biol.*, 55: 853-867.

Navabpour, S., Morris, K., Allen, R., Harrison, E., Mackerness, S. and Buchanan-Wollaston, V. 2003. Expression of senescence-enhanced genes in response to oxidative stress. *J. Exp. Bot.*, 54: 2285-2292.

Pontier, D., Gan, S., Amasino, R.M., Roby, D. and Lam, E. 1999. Markers for hypersensitive response and senescence show distinct patterns of expression. *Plant Mol. Biol.*, 39: 1243-1255.

Quirino, B.F., Normanly, J. and Amasino, R.M. 1999. Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defence-related genes. *Plant Mol. Biol.*, 40: 267-278.

Rauf, M., Arif, M., Dortay, H., Matallana-Ramírez, L.P., Waters, M.T., Gil Nam, H., Lim, P.O., Mueller-Roeber, B., Balazadeh, S. 2013. ORE1 balances leaf senescence against maintenance by antagonizing G2-like-mediated transcription. *EMBO Rep*. 14: 382-8.

Ren, G., An, K., Liao, Y., Zhou, X., Cao, Y., Zhao, H., Ge, X. and Kuai, B. 2007. Identification of a novel chloroplast protein AtNYE1 regulating chlorophyll degradation during leaf senescence in *Arabidopsis*. *Plant Physiol.*, 144: 1429-1441.

salt-promoted senescence. *Plant* J. 62: 250-64.

Schippers, H.M.J., Nunes-Nesi, A., Apetrei, R., Hille, J., Fernie, A.R. and Dijkwel, P.P. 2009. The Arabidopsis onset of leaf death5 Mutation of Quinolinate Synthase Affects Nicotinamide Adenine Dinucleotide Biosynthesis and Causes Early Ageing, *Plant Cell*, 20: 2909–2925.

Schippers, J.H.M., Jing, H.C., Hille, J. and Dijkwel, P.P. 2007. Developmental and hormonal control of leaf senescence. In: Senescence processes in plants, Vol. 26, Gan, S., ed, Blackwell Publishing. 145-170.

Shirzadian-Khorramabad R, Jing, H.C., Everts, G.E., Schippers, J.H.M., Hille, J. and Dijkwel, P.P. 2010a. A mutation in the cytosolic O-acetylserine (thiol) lyase induces a genome-dependent early leaf death phenotype in *Arabidopsis*, *BMC plant Biology*, 10:80.

Shirzadian-Khorramabad, R., Jing, H.C., Hille, J. and Dijkwel, P.P., A mutation in an *Arabidopsis* inositol polyphosphate 1-phosphatase delays senescence and deregulates stomatal aperture. 2008. In: Molecular Genetic regulation of leaf longevity; R. Shirzadian-Khorramabad, Groningen university, pp. 91-118.

Shirzadian-Khorramabad, R., Jing, H.C., Hille, J., Dijkwel, P.P. 2010b. Identification of *Arabidopsis* stay-green mutants with a functional ethylene-response pathway. In: Agronomy Society of New Zealand Special Publication No. l3 /Grassland Research and Practice Series No. 14, McGill and Rowarth ed., pp. 119-129.

Sturre, M.J.G., Shirzadian-Khorramabad, R., Schippers, J.H.M., Chin-A-Woeng, T.F.C., Hille, J., Dijkwel, P.P. 2009. Method for the identification of single mutations in large genomic regions using massive parallel sequencing. *Mol. Breeding*, 23:51-59.

Terce-Laforgue, T., Mäck, G. and Hirel, B. 2004. New insights towards the function of glutamate dehydrogenase revealed during source-sink transition of tobacco (*Nicotiana tabacum* L.) plants grown under different nitrogen regimes. *Physiol. Plant.*, 120: 220-228.

Thompson, A.R. and Vierstra, R.D. 2005. Autophagic recycling: lessons from yeast help define the process in plants. *Curr. Opin. Plant Biol.*, 8: 165-173.

Thompson, J.E., Froese, C.D., Madey, E., Smith, M.D. and Hong, Y. 1998. Lipid

metabolism during plant senescence. *Prog. Lipid Res.*, 37: 119-141.

Woo, H.R., Goh, C.H., Park, J.H., Teyssendier de la Serve, B., Kim, J.H., Park, Y.I. and Nam, H.G. 2002. Extended leaf longevity in the *ore4-1* mutant of *Arabidopsis* with a reduced expression of a plastid ribosomal protein gene. *Plant J.*, 31: 331-340.

Yang, S.D., Seo, P.J., Yoon, H.K. and Park, C.M. 2011. The Arabidopsis NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the COR/RD genes. *Plant Cell*, 23: 2155–2168

کنترل ملکولی- ژنتیکی پدیده طول عمر برگ در گیاهان

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چکیده

فرآیند پیری آخرین مرحله از رشد و نمو برگ گیاهان است. این فرآیند بر مبنای یک برنامه ژنتیکی معین در گیاهان آغاز شده و نهایتاً به مرگ سلولی می‌انجامد. هدف غایی از وقوع آن در گیاهان انتقال المنت‌های غذایی ماکرو و میکرو از برگ‌های در شرف پیرشدن به قسمت‌های جوان و در حال رشد گیاهان است. وقوع این فرآیند در برگ با تغییرات گسترده‌ای در بیولوژی حیات گیاه از جمله در بخش‌های فیزیولوژیکی، مورفولوژیکی، بیوشیمیایی و ملکولی گیاهان همراه است. در این راستا نتایج حاصله از مطالعه پروفیل بیان ژنهای وابسته به پیری و شناسایی و بررسی موتانت‌های مختلف پیری توانست بطور قابل توجهی بر شناخت ما در خصوص سیستم ژنتیکی کنترل‌کننده فرآیند پیری در گیاهان بیافزاید. در این مقاله مروری سعی شده به یاقته‌های مهم در زمینه ژن‌های موثر بر کنترل پیری برگ بر مبنای مطالعه موتانت‌های پیری در گیاهان مختلف و بخصوص گیاه آرابیدپسیس بعنوان مدل گیاهی بپردازد. لذا عوامل موثر بر تنظیم ملکولی فرآیند پیری بر مبنای مسیرهای بیوشیمیایی فعالیت آنها طبقه‌بندی شده و مورد بحث قرار گرفته‌اند.

کلمات کلیدی: فرآیند پیری، طول عمر، گیاه آرابیدپسیس.