# Leaf Senescence – An Overview

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***Review Article***

***Abstract:***Leaf senescence is highly complex and regulated process. In the last two decade, the main focus of research has been on expression of senescence associates genes (SAGs) as well as on the identification of senescence mutants. Analysis of these is to expand our understanding of the processes by which senescence functions. Presently the advances in genomics are providing scientists with a amazing array of tools for the identification and functional analysis of the genes and pathways involved in senescence. In this review, we have given general idea about leaf senescence process.

***Keywords:*** Plant, Leaf senescence, Senescence-associated gene, Gene regulation.

## Introduction:

Senescence is a process that takes place in a highly synchronized manner and the cell constituents are dismantled in an ordered succession. Chlorophyll degradation is the first observable symptom of senescence but by the time yellowing of the leaf can be seen, the majority of the senescence process has occurred. Leaf senescence is an important phase in the plant life, it involves degradation of macromolecules and mobilization of nutrients from the senescing leaf to the growing parts of the plant (Noodén, 1988). Leaf senescence progresses in an age dependent manner, but is also affected by a complex interaction of the developmental stage with various internal and outer factors (Nam, 1997). Internal factors include age and phytohormone levels; external factors include such as temperature, shading, light, drought, nutrient deficit and pathogen infection (Hensel *et al*., 1993). Fruit ripening and the seed development process also induce leaf senescence (Noodén and Guiamét, 1989).

Senescence-associated genes (SAGs) have been known in senescing leaves of dicot and monocot plants in the past years (Buchanan-Wollaston and Ainsworth,1997). These gene transcripts are either elevated from a basal level or are senescence-specific. The encoded gene products are principally involved in degradation or movement of nutrients, but they are also involved in protecting cell viability for targeting the senescence process. Many SAGs have functions that remain unidentified. Some are likely involved in triggering leaf senescence or controlling the progression rate of senescence (Chandalee, 2001).

## Process of leaf senescence:

The visible sign of senescence is yellowing of leaf, which is due to the degradation of chlorophyll molecules. The cycle for chlorophyll degradation has been elucidated by Matile *et al*., 1999, and a number of the genes in the pathway have been cloned. It has been reported that Senescence Associated Vacuoles (SAV) are involved in the degradation of the soluble photosynthetic proteins of the chloroplast stroma during leaf senescence process (Martinez *et al*., 2008). The chlorophyll degradation steps are not carried out in order to transport the nutrients, but take place to detoxify this highly reactive compound as it is released from the pigment–protein complexes. This is essential to maintain the viability of the plant cell while senescence is started. It is been reported by Hortensteiner, 2009 that stay green mutants delayed in leaf senescence process encode stay green genes, member of a new family of chloroplast located proteins, which

may have function in dismantling of chloroplast complexes in senescence process.

Proteins degradation is probably the most significant breakdown process that takes place during senescence since the remobilization of amino acids is very essential to supply the developing organs elsewhere in the plant. Several genes encoding different types of protease have been identified in senescing leaves from various plants. One of the most obvious enzymatic events that take place in the senescence process is proteolysis and it is therefore not surprising that there is de novo transcription of protease genes and synthesis of proteins. Increased levels of specific proteases in senescing leaves have been hard to notice reproducibly by biochemical methods due to the high level of proteases already present in the vacuole of the developing leaf part of plant (Feller and Fischer, 1994). It is not clear whether increased reactive oxygen species could start the early degradation of Rubisco during senescence process. Although reactive oxygen species levels do elevate during senescence, this is likely to be the result of macromolecule degradation processes and thus occur after protein and lipid degradation is initiated in senescence process. Some senescence enhanced proteases accumulate in the vacuole as an inactive aggregate, which slowly matures to produce a soluble active enzyme at later stages of senescence process (Yamada *et al*., 2001).

Lipid molecules which can be mobilized and used by the senescing leaf in senescence process. The level of total lipids level goes down in senescing leaves, membranes of the cell including the thylakoid membranes are metabolized to provide energy for the senescence process (Wanner *et al.,* 1991). Respiratory activity in a senescing leaf is elevated and a continuous energy supply is required to allow degradation and mobilization processes to take place.

The protein and lipid component of membranes are rich sources of nitrogen and carbon, it has been reported that during leaf senescence γ–aminobutyric acid (GABA)-transaminase play a key role in GABA shunt for the carbon and nitrogen metabolism (Ansari *et al*., 2005). During senescence, nitrogen which is present in proteins and nucleic acids molecules is converted to transportable amino acids, in particular the amides glutamine and asparagine, which are the predominant amino acid in phloem transported from senescence leaves (Feller and Fischer,1994). Membrane lipids are very rich source of phosphorus, and it is clear from studies of over wintering trees that the transfer of both nitrogen and phosphorus from senescing leaves to perennial tissues is important for plant fitness (Hoch *et al*., 2003). It has been reported that the delay of leaf senescence in wheat also delays the translocation of metabolites from leaves to developing seeds, as indicated by higher accumulation of ($^{15}$N-labelled) N in spikes of control compared with transgenic plants just before to anthesis (Sykorova *et al.,* 2008). Recently it has reviewed in detail regarding the nutrients recycling and developmental regulation during senescence (*Guiboileau et al.,* 2010). Variation in the sugar level also has been reported onset of senescence process (Doorn, 2008). During senescence, nucleic acids, in particular the rRNA, within a senescing cell are an important source of carbon, nitrogen and especially phosphorus. DNA level remain constant in a senescing cell as senescence progresses, but the levels of ribosomal RNA goes down (Makrides and Goldthwaite, 1981).

## Gene regulation during leaf senescence process:

Over two decades of biochemical and molecular studies over senescence process have shown that senescence is an active process that requires the expression of novel genes and the synthesis of new kind proteins. Genes with a role in leaf senescence process can be characterized by the isolation and characterization of mutants that are defective in some aspect of the senescence process pathway. Recent molecular studies support that the onset of senescence involves the expression of a complex array of genes and its products are involved in senescence, and are responsible for senescence-related biochemical and cellular changes (Andersson *et al*., 2004; Price *et al.*, 2008.). Guo *et al.* (2004) has provides comprehensive transcriptome study with 6,200 of senescence associated expressed sequence tags (ESTs), representing approximately 2,500 genes, in *Arabidopsis thaliana* leaves.

Signaling and transcriptional networks regulate the process where and when senescence is initiated. It has been reported that post-transcriptionally activated eukaryotic translation initiation factor 5A (eIF5A) plays a role in the post-transcriptional regulation of senescence process (Thompson *et al*., 2004). Plant as well as in animal eIF5A are post-translationally modified by two enzymes, deoxyhypusine synthase (DHS) and eoxyhypusine hydroxylase (DHH), which play role in the conversion of a conserved lysine residue to the unusual amino acid, hypusine. The hypusinemodified eIF5A is suppose to be the active form of the protein (Wang *et al*., 2001). Cytokinin-mediated phosphorylation of ARR2, an *Arabidopsis* response regulator, leads to trans-activation of cytokinin-responsive genes play role in increasing leaf longevity and delaying senescence (Kim *et al*., 2006). Transcription factor WRKY play a central role in leaf senescence process of *Arabidopsis thaliana* and WRKY53 is one of the transcription factor that evolved in early senescence process (Zentgraf *et al.,* 2010). Recently transcription factor NAAC092 has been reported that it play a central role in leaf senescence process of *A. thaliana* (Balazadeh *et al.,* 2010).

## Applications of leaf senescence in agricultural improvement:

It is very important for future agronomic improvements in many crop species after knowing the complete senescence process. Delaying senescence, particularly of the flag leaf, in seed crops such as wheat or maize would help to increase the grain yield and stay-green varieties are used in some cereal crop improvement (Borrell *et al*., 2001). Early senescence induced by stress also has a damaging effect on yield, and stay-green plants can exhibit improved stress resistance. In addition, the loss of quality caused by post-harvest induced senescence has a harsh effect on the shelf life of green vegetables. Delaying senescence would decrease wastage throughout the supply chain. Gan and Amasino (1995) reported that the transgenic tobacco plants that showed autoregulated synthesis of cytokinin from the *SAG12* promoter had an enlarged leaf number and seed yield. Study by Jordi *et al*. (2000), using nitrogen-limited growth conditions, showed that chlorophyll levels were maintained in senescing leaves on the transgenic plants but soluble protein levels and photosynthetic activity were not very different from wild-type. Information of the hormonal control of senescence, gained from leaf senescence studies in *Arabidopsis* and tomato, has been used to manipulate post-harvest senescence in other crops. Ethylene levels boost in broccoli florets post-harvest and the enhanced expression of genes encoding ethylene biosynthesis genes has been reported (Pogson *et al.,* 1995). Transgenic broccoli containing an antisense ACC oxidase gene showed major reduction in ethylene production and improvement in head colour changes after harvest of broccoli (Henzi *et al*., 2000).

## Conclusions:

Mechanism of leaf senescence has expanded rapidly in the last two decade. Novel discoveries have greatly improved our knowledge and understanding of senescence. From a practical perspective, massive annual losses in yield due to early senescence, post harvest senescence as well as pathogen and stress-induced premature senescence continue to threaten the commercial viability of many crops through out the world. Gene expression analyses of the senescence transcriptome established through microarray technique have provided significant insights into the nature and role of changes in gene expression during senescence process, but these technologies alone will not elucidate the complexities and interactivities of the molecular cascades underlying senescence. Our information of senescence process grows, it is becoming increasingly clear that a combination of physiological, biochemical, genetic and molecular approaches will be compulsory to fully elucidate the exquisite regulation of both its initiation and execution process of senescence.

## References:

[1] Andersson, A., Keskitalo, J., Sjödin, A., Bhalerao, R., Sterky, F., Wissel, K., Aspeborg, A., Moyle, R., Ohmiya, Y. and Bhalerao, R., A transcriptional

time table of autumn senescence. Genome Biolology. 5 : R24, 2004.

[2] Ansari, M. I., Lee, R. H. and Chen, S.C.G., A novel senescence-associated gene encoding γ-aminobutyric acid (GABA):pyruvate transaminase is upregulated during rice leaf senescence. Physiologia Plantarum. 123 : 1-8, 2005.

[3] Balazadeh, S., Wu, A., Roeber, B.M., Salt-triggered expression of the ANAC092 dependent senescence regulon in Arabidopsis thaliana. Plant Signalling and Behaviour. 5 : 733-735, 2010.

[4] Borrell, A., Hammer, G. and van Oosterom, E., Stay-green: a consequence of the balance between supply and demand for nitrogen during grain filling? Ann. Appl. Biol. 138 : 91–95, 2001.

[5] Buchanan-Wollaston, V. and Ainsworth, C., Leaf senescence in Brassica napus: cloning of senescence-related genes by subtractive hybridization. Plant Molecular Biology. 33 : 821-834, 1997.

[6] Chandlee, J.M., Current molecular understanding of the genetically programmed process of leaf senescence. Physiologia Plantarum. 113 : 1-8, 2001.

[7] Doorn, W.G., Is the onset of senescence in leaf cells of intact plants due to low or high sugar level? Journal of Experimental Botany. 59 : 1963-1972, 2008.

[8] Feller, U. and Fischer, A., Nitrogen metabolism in senescing leaves. Critical Reviews in Plant Sciences. 13 : 241-273, 1994.

[9] Gan, S. and Amasino, R.M., Inhibition of leaf senescence by autoregulated production of cytokinin. Science. 270 : 1966-7, 1995.

[10] Grbic, V. and Bleecker, A.B., Ethylene regulates the timing of leaf senescence in Arabidopsis. Plant Journal.. 8 : 595 602, 1995.

[11] Guo, Y., Cai, Z. and Gan, S., Transcriptome of Arabidopsis leaf senescence.Plant Cell and Environment. 27 : 521–549, 2004.

[12] Guiboileau, A., Sormani, R., Meyer, C., Daubresse, C.M., Senescence and death of plant organs: Nutrients recycling and developmental regulation. C.R. Biologies. 33 : 382-391, 2010.

[13] Hensel, L.L., Grbic, V., Baumgarten, D.A. and Bleecker, A.B., Developmental and age related processes that influence the longevity and senescence of photosynthetic tissue in Arabidopsis. Plant Cell. 5 : 553-564, 1993.

[14] Henzi, M.X., Christey, M.C. and McNeil, D.L., Morphological characterization and agronomic evaluation of transgenic broccoli (Brassica oleracea L. var. italica) containing an antisense ACC oxidase gene. Euphytica. 113 : 9–18, 2000.

[15] Hoch, W.A., Singsaas, E.L. and McCown BH., Resorption protection. Anthocyanins acilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiology. 133 : 1296–1305, 2003.

[16] Hortensteiner, S., Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends in Plant Sciences. 14 : 155-162, 2009.

[17] Jordi, W., Schapendonk, A., Davelaar, E., Stoopen, G.M., Pot, C.S., de Visser, R., van Rhijn, J.A., Gan, S. and Amasino, R.M., Increased cytokinin levels in transgenic PSAG12-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. Plant Cell Environ. 23 : 279– 289, 2000.

[18] 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., Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in Arabidopsis. Proceedings of the National Academy of Sciences, USA. 103 : 814–819, 2006.

[19] Makrides, S.C., Goldthwaite J., Biochemical changes during bean leaf growth, maturity and senescence. Contents of DNA, polyribosomes, ribosomal RNA, protein and chlorophyll. Journal of Experimental Botany 32 : 725-35, 1981.

[20] Matile, P., Hörtensteiner, S. and Thomas, H., Chlorophyll Degradation. Annual Review of Plant Physiology and Plant Molecular Bioliology. 50 : 67–95, 1999.

[21] Martínez, D. E., Costa, M. L., Gomez, F.M., Otegui, M.S. and Guiamet, J.J., "Senescence-associated vacuoles" are involved in the degradation of chloroplast proteins in tobacco leaves. Plant Journal. approved for publication "Postprint"; doi: 10.1111/j.1365-313X.2008.03585.x, 2008.

[22] Nam, H.G., The molecular genetic analysis of leaf senescence. Current Opinion in. Biotechnology. 8 : 200-207, 1997.

[23] Noodén, L.D., The phenomena of senescence and aging. In: Noodén LD, Leopold AC, eds. Senescence and aging in plants. Academic Press, San Diego, CA, 1-50, 1988.

[24] Nooden, L.D. and Guiamet, J. J., Regulation of assimilation and senescence by the fruit in monocarpic plants. Physiologia. Plantarum. 77 : 267-274, 1989.

[25] Price, A.M., Orellana, D.F., Salleh, F.M., Stevens, R., Acock, R., Buchanan Wollaston, V., Stead, A.D. and Rogers, H.J., A comparison of leaf and petal senescence in wallflowers (Erysimum linifolium) reveals common and distinct patterns of gene expression and physiology. Plant Physiology. Jun 6. approved for publication, 2008.

[26] Pogson, B.J., Downs, C.G. and Davies, K.M., Differential expression of two 1-aminocyclopropane-1-carboxylic acid oxidase genes in broccoli after harvest. Plant Physiology. 108 : 651–657, 1995.

[27] Sykorova, B., Kuresova, G., Daskalova, S., Trckova, M., and Hoyerov, K., Senescence-induced ectopic expression of the A. tumefaciens ipt gene in wheat delays leaf senescence, increases cytokinin content, nitrate influx, and nitrate reductase activity, but does not affect grain yield. Journal of Experimental Botany. 59 : 377–387, 2008.

[28] Thompson, J.E., Hopkins, M.T., Taylor, C. and Wang, T.W., Regulation of senescence by eukaryotic translation initiation factor 5A: implications for plant growth and evelopment. Trends in Plant Science. 9 : 174–179, 2004.
[29] Wanner, L., Keller, F. and Matile, P., Metabolism of radiolabelled galactolipids in senescent barley leaves. Plant Science. 78 : 199-296, 1991.
[30] Wang, T.W., Lu, L., Wang, D. and Thompson, J.E., Isolation and characterization of senescence-induced cDNAs encoding deoxyhypusine synthase and eucaryotic translation initiation factor 5A from tomato. Journal of Biological Chemistry. 276 : 17541–17549, 2001.
[31] Yamada, K., Matsushima, R., Nishimura, M. and Hara-Nishimura, I., A slow aturation of a cysteine protease with a granulin domain in the vacuoles of senescing Arabidopsis leaves. Plant Physiology. 127 : 1626–1634, 2001.
[32] Zentgraf, U., Laun, T., Miao, Y., The complex regulation of WRKY53 during leaf senescence of Arabidopsis thaliana. European Journal of Cell Biology. 89 : 133-137, 2010.